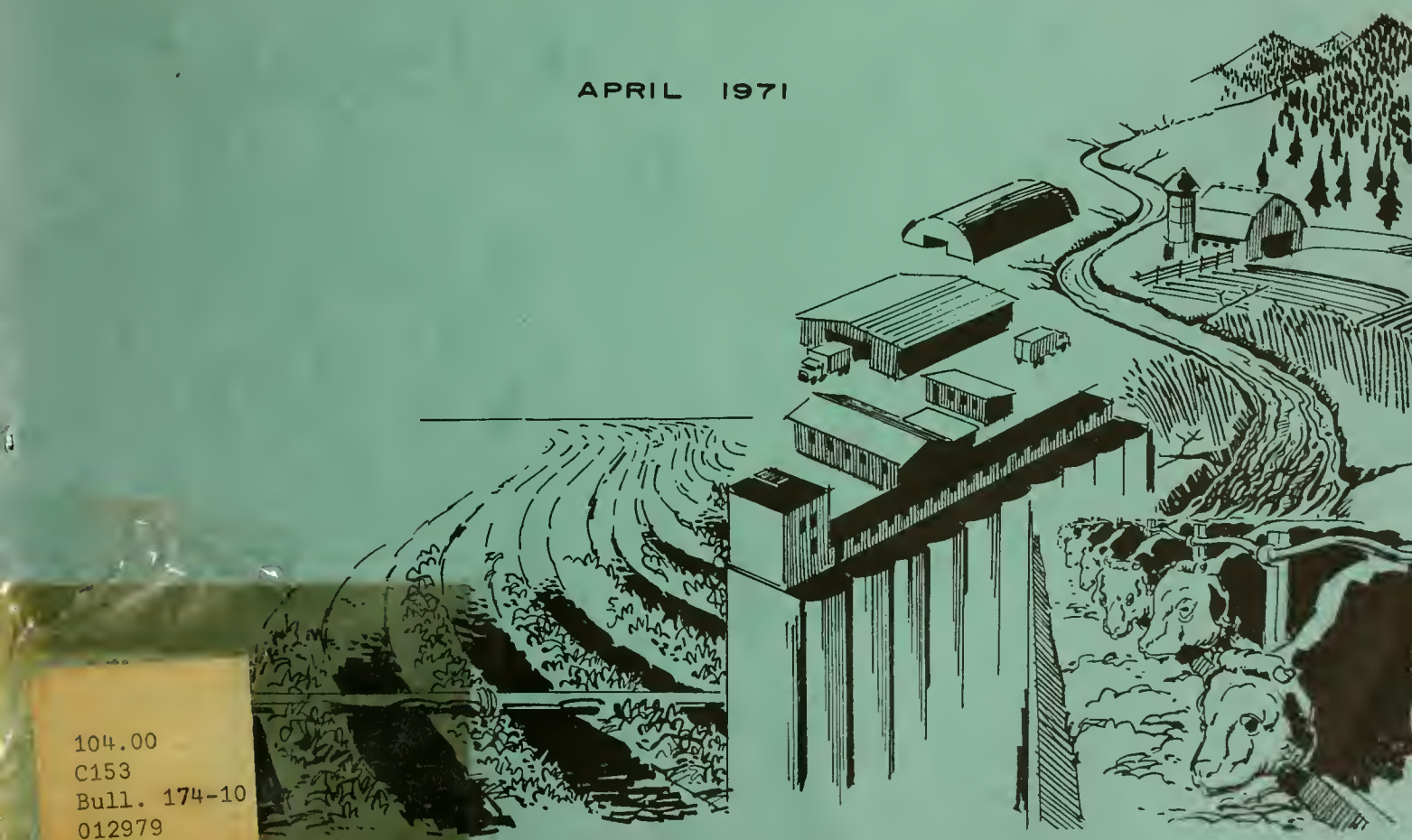
WATER POLLUTION CONTROL RESEARCH SERIES • 13030ELY4/71-7
REC-R2-71-7
DWR NO. 174-10

BIO-ENGINEERING ASPECTS OF AGRICULTURAL DRAINAGE
SAN JOAQUIN VALLEY, CALIFORNIA

REMOVAL OF NITRATE BY AN ALGAL SYSTEM

APRIL 1971



CALIFORNIA DEPARTMENT OF WATER RESOURCES

BIO-ENGINEERING ASPECTS OF AGRICULTURAL DRAINAGE SAN JOAQUIN VALLEY, CALIFORNIA

The Bio-Engineering Aspects of Agricultural Drainage reports describe the results of a unique interagency study of the occurrence of nitrogen and nitrogen removal treatment of subsurface agricultural wastewaters of the San Joaquin Valley, California.

The three principal agencies involved in the study are the Water Quality Office of the Environmental Protection Agency, the United States Bureau of Reclamation, and the California Department of Water Resources.

Inquiries pertaining to the Bio-Engineering Aspects of Agricultural Drainage reports should be directed to the author agency, but may be directed to any one of the three principal agencies.

THE REPORTS

It is planned that a series of twelve reports will be issued describing the results of the interagency study.

There will be a summary report covering all phases of the study.

A group of four reports will be prepared on the phase of the study related to predictions of subsurface agricultural wastewater quality -- one report by each of the three agencies, and a summary of the three reports.

Another group of four reports will be prepared on the treatment methods studied and on the biostimulatory testing of the treatment plant effluent. There will be three basic reports and a summary of the three reports. This report, "REMOVAL OF NITRATE BY AN ALGAL SYSTEM", is one of the three basic reports of this group.

The other three planned reports will cover (1) techniques to reduce nitrogen during transport or storage, (2) possibilities for reducing nitrogen on the farm, and (3) desalination of subsurface agricultural wastewaters.

BIO-ENGINEERING ASPECTS OF AGRICULTURAL DRAINAGE
SAN JOAQUIN VALLEY, CALIFORNIA

REMOVAL OF NITRATE BY AN ALGAL SYSTEM

Prepared by the

California Department of Water Resources
William R. Gianelli, Director

The agricultural drainage study was conducted under the direction of:

Robert J. Pafford, Jr., Regional Director, Region 2
UNITED STATES BUREAU OF RECLAMATION
2800 Cottage Way, Sacramento, California 95825

Paul DeFalco, Jr., Regional Director, Pacific Southwest Region
WATER QUALITY OFFICE, ENVIRONMENTAL PROTECTION AGENCY
760 Market Street, San Francisco, California 94102

John R. Teerink, Deputy Director
CALIFORNIA DEPARTMENT OF WATER RESOURCES
1416 Ninth Street, Sacramento, California 95814

DWR-WQO Grant #13030 ELY
DWR-USBR Contract #14-06-200-3389A
April 1971

For sale by the Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402 - Price $1.25

REVIEW NOTICE

This report has been reviewed by the Water Quality Office, Environmental Protection Agency and the U. S. Bureau of Reclamation, and has been approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Water Quality Office, Environmental Protection Agency, or the U. S. Bureau of Reclamation.

The mention of trade names or commercial products does not constitute endorsement or recommendation for use by either of the two federal agencies or the California Department of Water Resources.

ABSTRACT

An algal system consisting of algae growth, harvesting and disposal was evaluated as a possible means of removing nitrate-nitrogen from subsurface agricultural drainage in the San Joaquin Valley of California. The study of this assimilatory nitrogen removal process was initiated to determine optimum conditions for growth of the algal biomass, seasonal variations in assimilation rates, and methods of harvesting and disposal of the algal product. A secondary objective of the study was to obtain preliminary cost estimates and process design.

The growth studies showed that about 75 to 90 percent of the 20 mg/l influent nitrogen was assimilated by shallow (12-inch culture depth) algal cultures receiving 2 to 3 mg/l additional iron and phosphorus and a mixture of 5 percent C02. Theoretical hydraulic detention times required for these assimilation rates varied from 5 to 16 days, depending on the time of the year. The total nitrogen removal by the algal system, assuming 95 percent removal of the algal cells, ranged from 70 to 85 percent of the influent nitrogen.

The most economical and effective algal harvesting system tested was flocculation and sedimentation followed by filtration of the sediment. The algal cake from the vacuum filter, containing about 20 percent solids, was then air- or flash-dried to about 90 percent solids. The market value for this product as a protein supplement was estimated to be about $80 to $100 per ton.

Preliminary estimates indicate that the removal of nitrate from tile drainage by an algal system will cost about $135 per million gallons of treated water. This figure includes engineering and contingency costs and recovery of some cost by the sale of an algal product. The estimate will be refined at the end of operational studies to be completed in 1970.

Key words: Algae stripping, nutrients, tile drainage, nitrogen removal, treatment costs.

BACKGROUND

This report is one of a series which presents the findings of intensive interagency investigations of practical means to control the nitrate concentration in subsurface agricultural wastewater prior to its discharge into other water. The primary participants in the program are the Water Quality Office of the Environmental Protection Agency, the United States Bureau of Reclamation, and the California Department of Water Resources, but several other agencies also are cooperating in the program. These three agencies initiated the program because they are responsible for providing a system for disposing of subsurface agricultural wastewater from the San Joaquin Valley of California and protecting water quality in California's water bodies. Other agencies cooperated in the program by providing particular knowledge pertaining to specific parts of the overall task.

The ultimate need to provide subsurface drainage for large areas of agricultural land in the western and southern San Joaquin Valley has been recognized for some time. In 1954, the Bureau of Reclamation included a drain in its feasibility report of the San Luis Unit. In 1957, the California Department of Water Resources initiated an investigation to assess the extent of salinity and high ground water problems and to develop plans for drainage and export facilities. The Burns-Porter Act, in 1960, authorized San Joaquin Valley drainage facilities as part of the State Water Facilities.

The authorizing legislation for the San Luis Unit of the Bureau of Reclamation's Central Valley Project, Public Law 86-488, passed in June 1960, included drainage facilities to serve project lands. This Act required that the Secretary of Interior either provide for constructing the San Luis Drain to the Delta or receive satisfactory assurance that the State of California would provide a master drain for the San Joaquin Valley that would adequately serve the San Luis Unit.

Investigations by the Bureau of Reclamation and the Department of Water Resources revealed that serious drainage problems already exist and that areas requiring subsurface drainage would probably exceed 1,000,000 acres by the year 2020. Disposal of the drainage into the Sacramento-San Joaquin Delta near Antioch, California, was found to be the least costly alternative plan.

Preliminary data indicated the drainage water would be relatively high in nitrogen. The then Federal Water Quality Administration conducted a study to determine the effect of

discharging such drainage water on the quality of water in the San Francisco Bay and Delta. Upon completion of this study in 1967, the Administration's report concluded that the nitrogen content of untreated drainage waters could have significant adverse effects upon the fish and recreation values of the receiving waters. The report recommended a three-year research program to establish the economic feasibility of nitrate-nitrogen removal.

As a consequence, the three agencies formed the Interagency Agricultural Wastewater Study Group and developed a three-year cooperative research program which assigned specific areas of responsibility to each of the agencies. The scope of the investigation included an inventory of nitrogen conditions in the potential drainage areas, possible control of nitrates at the source, prediction of drainage quality, changes in nitrogen in transit, and methods of nitrogen removal from drain waters including biological-chemical processes and desalination.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

FIGURES

FIGURES (Cont'd)

FIGURES (Cont'd)

FIGURES (Cont'd)

TABLES

TABLES (Cont'd)

CHAPTER I - CONCLUSIONS AND SUMMARY

Conclusions

1. Algal growth and harvesting is a technically feasible method of removing nitrate-nitrogen from subsurface agricultural tile drainage in the San Joaquin Valley. This method can be used to reduce 20 mg/l of influent nitrogen to 3-5 mg/l which includes organic nitrogen remaining in unharvested cells.

2. Laboratory algal growth assays comparing algae-treated and untreated tile drainage mixed with potential receiving waters showed that treatment lowered the biostimulatory nature of the waste.

3. Preliminary cost estimates for the system described in this report are about $135 per million gallons. This figure is based on results of feasibility studies and may be revised after 1970 operational studies.

Summary

Tile drainage will support extensive algal growth, providing environmental conditions are optimum for such growth. The effect of several chemical and physical factors on algal growth were studied in laboratory and outdoor cultures. The following summary of the factors includes the best estimates of optimum levels for outdoor cultures.

1. Three nutrient additives appeared necessary to support sustained massive growth of Scenedesmus quadricauda in tile drainage. About 2 milligrams of phosphorus per liter of waste were needed regardless of season. The addition of 5 percent carbon dioxide was required during part of the year, as was 2 to 3 mg/l of iron.

2. Mixing data obtained before carbon dioxide was added routinely to the outdoor cultures consistently showed that rapid mixing was required for maximum nitrogen removal. During the time when additional carbon dioxide was a growth requirement, ponds with carbon dioxide addition only had nitrogen assimilation rates comparable to those with both carbon dioxide and mixing. The exact mixing requirements of an algal system have not been defined but it appears that the maximum requirement will be about four hours of mixing at an average velocity of about 0.25 feet per second.

3. When phosphorus was available to the algae, detention time was normally the most important variable studied in individual outdoor culture experiments. The theoretical hydraulic detention times required for maximum nitrogen assimilation varied from 5 to 16 days and appeared to be directly related to pond temperature within the range of 12°C and 25°C, and independent of temperature within the range of 25°C to 33°C. As shown in items 4 and 5, depth and biomass control also significantly affected detention time.

4. The optimum culture depth in these studies was eight inches, but by a three to four day increase in detention time, comparable nitrogen assimilation rates could be obtained at a 12-inch depth. Comparison of the difference between nitrogen assimilation at two depths, 8 and 16 inches, showed that the difference varied seasonally and was directly related to available light. That is, depth had less effect during summer light conditions than during the winter months.

5. Some mechanism may need to be incorporated into the growth units to control algal biomass. This mechanism may consist of a settling area in the pond itself. The settling area would remove the heavier, older, and less metabolically active cells from the system. With such a system, a minimum summer detention time of five days at a 12-inch depth should be feasible. Without it, about eight days detention time may be needed.

The two series of laboratory studies which compared rates of nitrogen removal in effluents from various tile systems in the Valley showed that the Alamitos sump water used in these studies provided results comparable to the remaining systems studied. These studies also showed that the addition of iron, phosphorus, and carbon dioxide was required for maximum nitrogen assimilation by algae grown in water from any of the systems studied.

During the investigation relatively little definitive work was accomplished on soil-lined ponds. This process probably involves a combination of algal and bacterial metabolic pathways. Data from the two soil ponds at the IAWTC showed that removal efficiencies often were comparable to those of the best algal stripping pond and required only the addition of phosphorus to achieve these results.

Harvesting of algal biomass is divided into three steps or stages, namely, concentration, dewatering, and drying. These steps differ in the amount of moisture remaining in the algal product. Studies at the Interagency Wastewater Treatment Center demonstrated that algae can be readily separated from

agricultural tile drainage and concentrated to 1 to 2 percent solids (by weight) either by coagulation-flocculation and sedimentation with any of several chemical coagulants or by use of a rapid sand filter (Sanborn filter) with backwashing. The slurry resulting from the concentration process can then be dewatered to about 10 to 20 percent solids by vacuum filtration or by self-cleaning centrifugation. Using these processes, the effluent algae concentration will be only 5 to 10 percent of the influent, at least within the influent range of 100-600 mg/l of algae.

Laboratory jar tests were conducted to determine the effectiveness of various mineral coagulants (lime, alum, and ferric sulfate) to determine their effectiveness in achieving coagulation and flocculation of the alga Scenedesmus in growth pond samples. The studies showed that the additions of these minerals could effect 90 to 95 percent removal of the algae (influent suspended solids concentration of 100 to 600 mg/l) during all seasons of the year; however, the required concentrations varied with changes in operation of the growth unit. When iron ($FeCl_3$) was added to the rapid growth pond as an algal nutrient, the concentration of the reagents required to remove 90 to 95 percent of the algae was about 5 mg/l for ferric sulfate, 20 mg/l for alum, and 40 mg/l for lime. These are compared to about 80 mg/l, 100 to 140 mg/l, and 180 to 200 mg/l for the same compounds when iron was not present in the growth pond.

Approximately 60 polyelectrolytes were tested alone and with the mineral coagulants to evaluate their effectiveness in algal separation. Of the compounds tested, both anionic and cationic, 17 polyelectrolytes were found to aid coagulation and to be economically comparable to mineral coagulants. With iron added to the growth unit and no carbon dioxide addition, almost complete (99 percent) algal separation was obtained with less than 0.2 mg/l of the cationic polyelectrolyte, Cat-Floc.

The algal product was usually air dried to about 90 percent solids, although one sample was dried by a De Laval spray drier at the company's test facilities. Two to three days air drying was normally required to reduce the moisture content to the desired level.

Literature review and market predictions indicate that a market can probably be developed for an algal product that will retail at about $80 to $100 per ton. The question of marketability can be answered more completely when the evaluations of the algal product are obtained from the companies that received representative samples. The high ash content of dried algae (30 to 50 percent) from a nitrogen

removal plant operated on tile drain effluent may preclude its use as a food for livestock but not as a protein supplement for fowl, or as a soil conditioner. Modification of the harvesting process to include in-pond settling, as well as chemical coagulation-flocculation may result in the production of two different by-products, one with 10 percent ash, and the other with about 50 percent ash.

CHAPTER II. INTRODUCTION

This is the final report of field studies conducted by members of the Interagency Nitrogen Removal Group on the feasibility of using algal growth and harvesting (algae stripping) as a method of removing nitrate-nitrogen from subsurface agricultural drainage in the San Joaquin Valley. These studies were conducted at the Interagency Wastewater Treatment Center (IAWTC) located near Firebaugh, California. Field work began in January 1968 and continued through December 1969; however, operational studies will be conducted through December 1970. They will be discussed in a later (June 1971) report.

The original impetus for considering the algal process came from a formal feasibility report submitted to Department of Water Resources (DWR) (Oswald, et al 1964). Based on laboratory culture studies and a review of pertinent literature, this panel of consultants concluded that algae stripping was a technically feasible means of removing nutrients from tile drainage and that removal of these nutrients would reduce the potential of the drainage for causing deleterious algal blooms in the receiving waters. The consultants proposed that a two-stage field study be initiated to determine the exact levels of nitrogen removal which could be realized by the process. The first stage involved construction and operation of a small (50 x 200 feet) prepilot plant. If the results of the prepilot studies show that algal stripping is a promising method, two pilot plants, each having a capacity of one million gallons per day (mgd), would be built. The prepilot plant was designed by engineers of the California Department of Water Resources following suggestions by the consultants and construction was started in July 1967.

Algae stripping is an assimilatory removal process in which the nutrient in question is first incorporated into cellular tissue and the cells then removed from the medium. The process includes three distinct areas of activity: growing the algae, separation of the algae with the incorporated nutrient from the liquid phase (including drying of the algae), and disposal of the algal product. The effluent nitrogen from an algae stripping plant will consist of two fractions -- the influent dissolved nitrogen not assimilated by the algae and the particulate cellular nitrogen not removed by the separation process. At the IAWTC the effluent total nitrogen limit was 2 milligrams per liter (mg/l) as recommended by the 1967 Environmental Protection Agency (formerly the Federal Water Quality Administration) report entitled "San Joaquin Master Drain,

Effects on Water Quality of San Francisco Bay and Delta". This amounts to a 90 percent reduction of the 20 mg/l average nitrogen concentration predicted for a combined San Joaquin Valley tile drain effluent. To remove 90 percent of 20 mg/l by the algal process will require 95 percent assimilation by the algae, with subsequent removal of 95 percent of the algal biomass.

The concept of using algal growth to remove nutrients from wastewaters is not original with this project, but its application to agricultural wastewaters has received little consideration. Oswald and Gotaas (1957), Fitzgerald (1960), Hemens and Mason (1968) and North American Aviation (1967) are some of the workers reporting on the use of the algal process to remove nutrients, mainly nitrogen and phosphorus, from secondary sewage effluent. These studies all indicated that tertiary treatment by photosynthetic algae effectively removed nutrients but that some problems were encountered. Because algal growth is light and temperature dependent, growth and nutrient assimilation were reduced during periods of cloud cover and/or cold weather. The practical problem of economically removing the suspended algae was also noted. This investigation was intended to determine if these limitations were equally important in an area of moderate climate and with agricultural wastewater as the culture medium.

The general objective of the feasibility phase of this study was to determine whether algae stripping would effectively remove nitrogen from agricultural tile drainage effluent. In working towards this objective, several questions had to be answered before a true assessment could be obtained. Among the most important of these questions were:

1. Will agricultural tile drainage support sustained algal growth?

2. What levels of nitrogen assimilation can be expected from this growth?

3. What environmental conditions are needed for maximum nitrogen assimilation and growth?

4. Can the algae be readily and economically separated from the liquid phase?

5. What levels of total nitrogen removal can be obtained by the algal stripping process?

6. Is there a potential market for an algal product, or how do you dispose of the algal product?

7. What is the total cost of an algal treatment system, including growth, harvesting, and disposal?

8. Does the process do the intended job; that is, does the treated effluent have less potential for causing noxious algal blooms in the receiving waters?

9. Are the results obtained from an isolated tile system applicable to a combined drainage facility?

The discussion of the results of a study of the algae stripping process requires a basic understanding of various concepts of algal growth and separation. A brief review of some of the more pertinent facets of these two areas will be included in the following sections.

Algal Growth

Algae cells contain a group of pigments, the chlorophylls, which enable the organism to produce organic material through a series of reactions requiring light energy, water, carbon dioxide, and various inorganic nutrients. The photosynthetic process can be summarized by the following approximation (Jewell and McCarty, 1968):

$$aCO_2 + cNO_3^- + ePO_4^{-3} + (c+3e)H^+ + 1/2(b-c-3e)\ H_2O +$$

$$\text{sunlight} \longrightarrow C_aH_bN_cO_dP_e + (a+b/4+5c/4-d/2+5e/4)O_2$$

as indicated by this equation, the rate of nitrogen (or phosphorus) assimilation by algal cells is a function of the rate at which organic material is synthesized. In a nutrient removal system, such as that studied at the IAWTC, the ultimate goal of systems design is to have the undesirable nutrient -- nitrogen for example -- to limit formation of cellular material. Using this concept, all other required elements of photosynthesis should be available in optimum or excess amounts. To accomplish this goal, the effect of various factors on photosynthesis must be understood.

Growth Characteristics

Although this category does not fit conveniently into the classification of factors affecting photosynthetic activity, some explanation of algal growth in two general types of algal systems may be helpful. A generalized algal growth curve for algae grown in a culture without nutrient replenishment (batch culture) is shown in Figure 1. The initial period of adjustment to the medium (lag phase) is followed by a period of rapid cell division (log or exponential phase). Cell growth is eventually limited by nutrient availability or by light limitation caused by mutual cell shading. The algal biomass, or cell numbers, may then decline, although in multi-algal cultures the original species may be replaced by another alga with different nutritional requirements. The specific growth rate of cells during the exponential (log) growth phase in this type of system is a function of cell concentration and can be described by the equation:

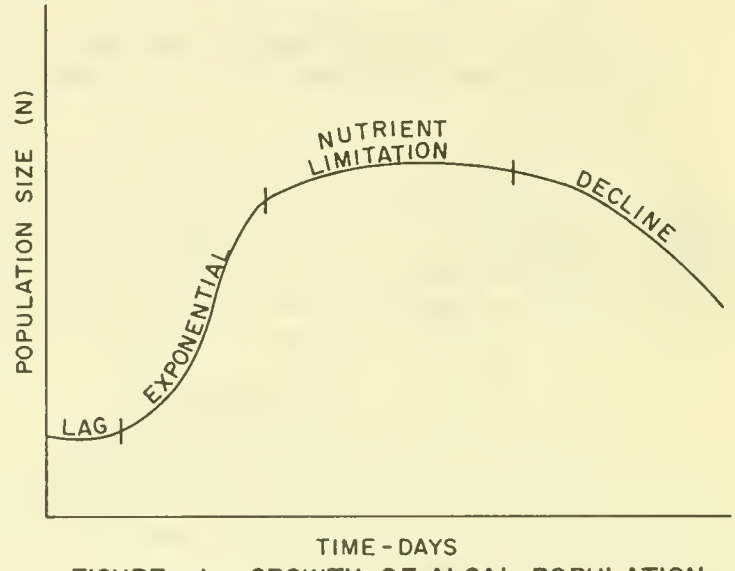


FIGURE 1 - GROWTH OF ALGAL POPULATION IN BATCH CULTURE (HYPOTHETICAL)

$$\frac{dN}{dt} = KN$$

where K is the specific growth rate (day^{-1}), N is the cell concentration (in any applicable unit), and t is the time in days.

In most waste treatment systems utilizing algae, algal nutrients flow continuously through the system and thus are constantly renewed. The theoretical analysis of this type of cellular growth, continuous culture, has been comprehensively treated by Monad (1949) and its application to algal systems reviewed by Retovsky (in Malek and Fencl, 1966) and Shelef, et al (1968). The importance of this type of process to algal cultures is that by manipulation of environmental characteristics (nutrient concentration,

light, and so forth) the algal population can be maintained at steady-state, or constant, population density. Conversely, manipulation of population density can cause some factor (nutrient) to be rate-limiting. As will be shown in the Materials and Methods chapter, the growth units used in this study were designed to be approximations of a continuous-flow type of system, with nitrogen as the rate-limiting factor.

Light

Absorption of light energy by dense algal cultures follows the Beer-Lambert law:

$$I_d = I_o e^{-Ecd}$$

where I_o is the incident light intensity, I_d is the intensity of light at any depth, d is depth in centimeters, c is the algal biomass in mg/l, E is the extinction coefficient in cm^2/mg, and e is the base of natural logarithms. As shown by this equation, light penetration to I_d is directly affected by incident light and inversely affected by depth and culture density. Optimum light intensities for maximum algal growth range from 200 to 400 foot-candles (ft-c), and the lower limit may be 100 ft-c. Using an extinction coefficient of 2×10^{-3} cm^2/mg (determined experimentally), Bogan, et al (1960) calculated the depth at which various incident light levels would penetrate several different concentrations of algae and still leave 100 ft-c. These values are shown in Table 1.

TABLE 1

RELATION BETWEEN INCIDENT LIGHT INTENSITY, DEPTH AND ALGAL CONCENTRATION AT I_d = 100 ft-c
(From Bogan, 1960)

Algal Concentration (mg/l)	Depth - cm for corresponding I_o			
	1,000 ft-c	2,000 ft-c	5,000 ft-c	10,000 ft-c
50	23	30	39	46
100	11.5	15	19.5	23
200	5.8	7.5	9.8	11.5
400	2.9	3.8	4.9	5.8

Using similar calculations Golueke and Gotaas (1958) determined that 4.5 inches was the theoretical optimum pond depth for domestic wastes, but practical experience indicated that optimum culture depth below which light no longer limited algal growth was about 8 inches.

The practical implications of the light-absorption equation are that if light-limiting conditions are to be avoided, high-rate algal ponds must be shallow and that depth may have to be adjusted seasonally. Even in the relatively shallow cultures used in chemostat (laboratory continuous-flow) studies, only a small percentage of the incident light energy is converted to cellular energy. Oswald (1963) reported that in laboratory studies with settled sewage, an average of 4 percent of the incident energy was fixed by the algal cultures. Conversion efficiency varied inversely with intensity, duration of light, and detention time, and directly with temperature and carbon dioxide concentration.

Another possible method of increasing the availability of incident light to individual cells is to move the algae into the light path by induced turbulence (mixing). Kok (1953) found that increased algal yield could be obtained in Chlorella cultures by using intermittent or flashing light, and that the optimum proportion of light-to-dark period appeared to be about 1:9. In these studies the flash time varied from 3 to about 200 milliseconds. In outdoor cultures the flashing light effect can be achieved by mixing a sufficiently dense algal culture; however, the mixing would have to be such that the duration of exposure to light and dark were of the correct time intervals. Mixing of this type would not be economically feasible in conventional wastewater treatment systems.

Temperature

As with all organisms, temperature affects the growth rate of algae, normally following the Van't Hoff rule according to which the growth rate doubles for each 10°C increase in temperature, within the range of temperature tolerance. Oswald (1963), working with Chlorella pyrenoidosa, noted that optimum light conversion efficiency occurred at 20°C. Using mixed cultures of Chlorella and Scenedesmus, Witt and Borchardt (1960) found that the light saturation intensity level (lowest light level at which maximum growth rate was attained) was directly affected by temperatures between 20 and 30°C. Because it is normally impractical to heat an outdoor algal culture, seasonal temperature variations cause changes in the required detention times of the system. In periods of cold weather, the algae

are allowed a longer contact time with the nutrient supply, which compensates for slower growth rates.

Carbon Source

Algae normally use free carbon dioxide as an inorganic carbon source, although some algae have been reported to use the bicarbonate ion. Osterlind (1950) stated that Scenedesmus quadricauda could freely use the bicarbonate ion but that Chlorella pyrenoidosa would only grow if provided with carbon dioxide (CO_2). In poorly buffered systems, use of CO_2 and bicarbonate (HCO_3^-) causes the equilibria in the following equations to shift to the right, accompanied by a rise in pH.

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \qquad H^+ + HCO_3^- \rightleftharpoons H^+ + CO_3^=$$

The concentration of any of the components of the carbon dioxide-bicarbonate-carbonate buffer system is a function of temperature, pH, and total dissolved solids as well as the concentrations of the remaining components. The equilibrium equation for the formation of hydrogen and bicarbonate ions from carbonic acid is:

$$\frac{\left[HCO_3^- + H^+\right]}{\left[H_2CO_3\right]} = K_1$$

where the dissociation constant, K_1, has been reported to be 3.5×10^{-7} at 18°C (Chemical Rubber Publishing Co., 1951). At a pH of 8 the ratio of carbonic acid to bicarbonate ion is 0.0286, at a pH of 7 it is 0.286, and at a pH of 6 it is 2.86 (McKee and Wolf, 1963). A similar equilibrium reaction between HCO_3^- and $CO_3^=$ has a reported K2 of 4.4×10^{-11} at 25°C (Chemical Rubber Publishing Co., 1951); thus at pH 7 the ratio of bicarbonate to carbonate ions would be 2,270 to 1, whereas at pH 11 the ratio would be 1 to 4.4 (McKee and Wolf, 1963).

At pH values above 9, carbonate precipitates as calcium and magnesium salts, thus decreasing total alkalinity. These precipitates also remove many algal nutrients, especially phosphorus and heavy-metal trace elements, by forming complex salts. In outdoor algal cultures exposed to the atmosphere, afternoon pH values may be as high as 10.5 to 11.

Inorganic Macronutrients

Inorganic macronutrient requirements include nitrogen and phosphorus. Most algae containing chlorophyll are able to use either ammonia or nitrate as a nitrogen source, and many species can use nitrite providing the concentration is low, about 0.001 molar (Fogg and Wolfe, 1954). Ammonium-N has been reported to be used in preference to nitrate, when both sources are provided to the same culture (Schuler, et al, 1953). As shown by the following equations, the use of either of these nitrogen sources can cause undesirable changes in culture pH (Cramer and Meyers, 1948).

$$1.0(NO_3^-)+5.7(CO_2)+5.4(H_2O)\rightarrow C_{5.7}H_{9.8}O_{2.3}N_{1.0}+8.25\ O_2+1.0(OH^-)$$

$$1.0(NH_4^+)+5.7(CO_2)+3.4(H_2O)\rightarrow C_{5.7}H_{9.8}O_{2.3}N_{1.0}+6.25\ O_2+1.0(H^+)$$

Nitrate assimilation results in the production of OH^- ions which causes a rise in pH; whereas ammonium assimilation lowers the pH by formation of hydrogen ions.

In addition to dissolved nitrogen sources, many blue-green algae can fix atmospheric nitrogen (Fogg, 1947). A requisite for nitrogen fixation appears to be an extremely low concentration of available dissolved nitrogen in the growth medium. Dominance of nitrogen-fixing algae in a treatment plant would preclude the use of algae stripping as a means of removing nitrogen.

Algae usually use phosphorus as orthophosphate (PO_4^{-3}). The ratio of nitrogen to phosphorous concentrations in a typical algal cell is about 10:1. Phosphorus is essential to algal growth. Without it, no growth will occur, regardless of the algal species. Sawyer (1952) found that the N:P ratios in natural waters that were seemingly optimal for algal blooms varied from 30:1 to 15:1, depending on the algal species involved. Some algae, when provided with quantities of phosphorus in excess of their requirements, can "store" the element up to a certain concentration (Ketchum, 1939a). Zabat (1970) found that, regardless of the availability of phosphorus, the maximum uptake by Chlorella sorokiawa was 2 percent of the cellular dry weight. Phosphorus uptake can be influenced by light (Gest and Kamen, 1948) and hydrogen ion concentration. Hydrogen ion concentration affects availability and has been mentioned previously.

Micronutrients

Many trace minerals are required for algal growth, although the actual concentrations required may be beyond the limit of detection by routine analytical procedures. The elements most commonly mentioned are iron, molybdenum, manganese, vanadium, cobalt, zinc, copper, sodium, and boron. In laboratory cultures, an accepted method of providing these nutrients is by means of a soil extract (the supernatant from a sample of soil boiled in distilled water). The trace element concentration in agricultural tile drainage may resemble a soil extract in that the water passes through several feet of soil before entering the drainage system.

This brief summary shows that the growth studies at the IAWTC had to include the investigation of several variables, some of which (temperature and light, for example) were fixed by natural conditions.

Algal Harvesting

This phase of the study of the algae stripping process was designed to determine the most feasible method of removing the algae from the growth units' effluent while maintaining an acceptable concentration of algae in the plant effluent. The harvesting process can be divided into three distinct areas of activity, based on the amount of water retained in the algal product. The first step, concentration, increases the solids from 0.015 to 0.040 percent by weight (depending on the concentration of algae in the growth unit) to 1 to 4 percent. The second step is dewatering which then brings the solids to 8 to 20 percent, and finally in the third step, the algal mass is dried to 85 to 92 percent solids by weight. At the latter moisture content, algae can be stored almost indefinitely without decomposition (Oswald and Golueke, 1960).

Relatively little work has been done on algal separation, especially at prepilot or pilot-scale levels. Oswald and Golueke (1968) present a comprehensive review of the results of several years of work on the problem of separating microscopic algae grown on secondary sewage effluent. In general, their results showed that the algae could be most economically concentrated by coagulation, flocculation, and sedimentation. (Coagulation is defined as the destabilization of colloidal particles, and flocculation as the mixing of the destabilized particles to encourage the formation of larger "floc" masses.) Dewatering was accomplished

by centrifugation, with final drying in the open. An alternative to separate dewatering and drying steps was to spread the concentrated algal slurry on sandbeds, which brought about the desired moisture content without the intermediate step of dewatering. North American Aviation (1967) also found sandbed dewatering and drying feasible in the harvesting of sewage-grown algae. In their studies, the algae were concentrated by sedimentation after coagulation and flocculation and then spread on sandbeds. These studies were conducted in Central and Southern California, which are areas of moderate climate.

A primary consideration in the algal harvesting process is the concentration of algae remaining after routine separation of the algal biomass from the liquid phase. Based on 2 mg/l total nitrogen in the effluent, the maximum allowable concentration of algae in the effluent would be about 15 mg/l. The 2 mg/l effluent nitrogen will probably contain about 0.5 mg/l dissolved nitrogen with the rest as particulate nitrogen. Algae contain about 10 percent N; therefore, 15 mg/l of algae can provide up to 1.5 mg/l N. Based on these figures, about 90 to 95 percent of the algae would have to be removed from the effluent of an operating algal treatment plant. Without this level of removal, organically-bound nitrogen would be regenerated by bacterial decomposition of the algae and would be available for algal growth downstream from the treatment site. Jewell and McCarty (1968) found that the amount of nitrogen and phosphorus regenerated by the aerobic decomposition of algae varied with culture age, with almost no regeneration taking place in older cultures. The average amount of N regenerated after about 300 days of aerobic decomposition was about 49 percent of the original amount present in the cells. Foree and McCarty (1968) reported that after 200 days of anaerobic decomposition, algal cultures retained about 60 percent of the original particulate nitrogen. These studies indicate that algae cellular nitrogen must be considered to be part of the total nitrogen released from a nitrogen stripping plant.

CHAPTER III - MATERIALS AND METHODS

The studies described in this report, with the exception of regrowth studies, were conducted at the Interagency Wastewater Treatment Center. In both algal growth and harvesting, studies were conducted using laboratory "bench-scale" experiments and expanded prepilot and pilot-scale studies. In general, the laboratory tests in both phases of the algal system were designed to screen a series of compounds or variables before testing in the larger systems. The following sections describe in detail the various materials and methods.

Laboratory - Chemical and Biological

A chemical and biological laboratory was located at the site and contained the equipment needed to perform routine chemical analyses. This included a Kjeldahl distillation and digestion unit, gas chromatograph, specific ion electrodes, spectrophotometer and other routine laboratory equipment. For special analyses, samples were sent to the Department of Water Resources' laboratory at Bryte, California. Samples for trace metal analysis were sent to the U. S. Geological Survey laboratory in Sacramento for analysis by emission spectrograph. Almost all chemical analyses used in these studies followed the procedures outlined in Standard Methods for Examination of Water and Waste Water, American Public Health Association, 1965. Table 2 lists the routine chemical analyses used and the normal frequency of analysis.

As shown in Table 2, the analytical emphasis was on nitrogen forms, especially nitrate and nitrite. Preliminary studies on all the listed constituents indicated that sampling and analysis at a greater frequency than shown was not necessary to follow changes in the algal system. Most of the analyses listed were completed within two to five hours of sample collection and were not preserved in any way.

TABLE 2

CHEMICAL ANALYSIS SCHEDULE

Constituent	Frequency	Method
Nitrate	3 times/wk.	Brucine, specific ion electrode
Nitrite	3 times/wk.	Diazotization
Ammonia	once/wk.	Kjeldahl - Distillation
Organic Nitrogen	twice/wk.	Kjeldahl
Orthophosphate	twice/wk.	Stannous chloride
Iron	once/wk.	Phenanthroline
Chemical Oxygen Demand	Occasionally	Dichromate refluxing
Dissolved Oxygen	Occasionally	Winkler-Azide Modification
pH	Daily	Electrode
Alkalinity	twice/wk.	Titration - pH meter
Electrical Conductivity	Occasionally	Wheatstone Bridge
Total Dissolved Solids	Occasionally	Evaporation

Precision and accuracy tests on all nitrogen forms and orthophosphate indicated that experimental error was within the limits suggested in Standard Methods. In spite of this, the routine use of the brucine test for nitrate, with some procedural modifications, often gave results of questionable validity. To avoid this problem, use of the nitrate specific ion electrode was begun early in 1969. The instrument was standardized against known concentrations of nitrate in denitrified drainage water. A plot of meter readings versus concentration showed a straight line between 0.5 and 50 mg/l N. Although some problems were encountered, mainly day-to-day variations in electrode response, the specific ion electrode proved to be a rapid (up to 150 analyses per hour), simple, and reliable method of nitrate analysis.

The biology section contained microscopes, an incubator, bacterial culturing equipment, and other necessary biological supplies and equipment. The primary method used to determine changes in algal biomass was measurement of volatile suspended solids. Volatile suspended solids were normally run two to three times per week, with total suspended solids once a week. One of the volatile solids analyses was conducted on the day total organic nitrogen analyses were made. The procedure for suspended and volatile

solids involved filtering 50 to 100 milliliters of algal suspension onto a weighed, preignited Whatman GFA glass filter disk. The disk was then dried for one hour at 103°C, weighed, and ignited at 560°C for 15 minutes. A final weighing was then made to determine volatile solids, which were assumed to represent algal biomass.

Approximate cell counts were made at weekly intervals, using a hemacytometer counting cell. These cell counts were used mainly to observe the condition of the algae and changes in species composition.

In some lightbox studies, cell growth was followed by measuring *in vivo* chlorophyll fluorescence. A Turner Model III fluorometer was modified by adding a blue light source and the proper combination of filters (Corning CS 5-60 primary and a Corning CS 2-60 secondary) for measurement of chlorophyll a.

The fluorometer, with a different light source and filter combination, was also used to determine true detention times in the miniponds. Changes in effluent concentration of Rhodamine B dye were followed and the detention time and mixing characteristics of the unit determined.

Adjacent to the laboratory a continuous recording analyzer monitored rapid growth pond (RGP) water temperature and pH, and sunlight. A weather station, located on one of the RGP baffles, was checked daily to determine maximum and minimum air and water temperatures, evaporation, precipitation, and wind.

Algae

The original inoculum for the rapid growth pond was obtained in January 1968 from the University of California's Richmond Field Station. Approximately 100 pounds (20 percent solids) of a sewage-grown green alga, *Scenedesmus quadricauda*, or closely related species, were added directly to the RGP. This amount was added to the RGP on two more occasions, in the summer of 1968 and early in 1969 when other algal species became dominant in the pond cultures. During the remainder of 1969, *Scenedesmus* retained its dominance and the RGP was used as a source of inoculum for all growth studies.

A coenobium of typical Scenedesmus quadricauda cells is shown in Figure 2. This alga is normally a four-celled coenobium; however, it is also found in a single-cell form or with as many as 32 cells in a colony. The genus has a cosmopolitan distribution, and one or more of the 30 known freshwater species can be found in most freshwater environments in this country. The choice of this alga was mainly a matter of its availability in large quantities from the Field Station. Preliminary culturing studies using tile drainage demonstrated that the species would grow in this type of medium. Additionally, the use of Scenedesmus quadricauda has some inherent advantages over most other algal species. Because of its wide distribution, planktonic nature, and ease of culturing, much literature is available on its physiology and biochemistry. Also, this alga has been reported to use the bicarbonate ion (Osterlind 1950), a pathway not available to all algae.

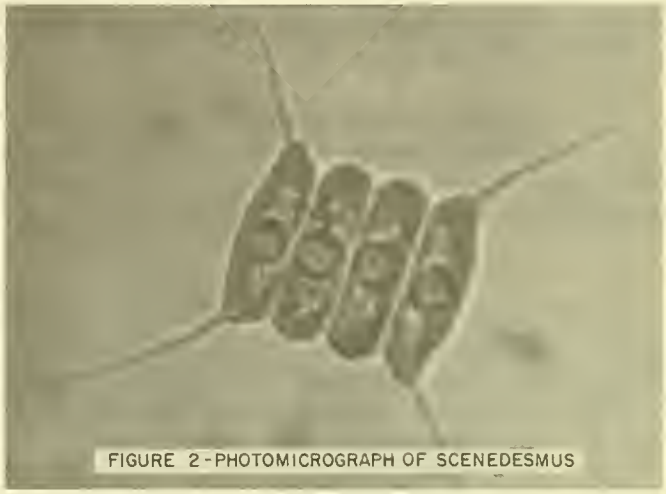


FIGURE 2-PHOTOMICROGRAPH OF SCENEDESMUS

Water

Subsurface agricultural drainage was obtained from a collecting system called the Alamitos tile system, located near the site. The Alamitos system was selected for its easy access from readily available Bureau of Reclamation land and because extensive sampling had indicated that the water quality was similar to that predicted for the combined drain. This particular tile system drained about 400 acres of intensively farmed land on which safflower or barley was grown in the winter and principally rice and cotton in the summer. This type of farming resulted in predictable changes in the quantity and quality of the drainage water, the effects of which are illustrated in Figure 3. Decreases in nitrate-nitrogen and total dissolved solids (TDS) concentration in the late spring were caused by increased tile flows when the rice crop was flooded. Tile flows varied from more than 1,000 gallons per minute (gpm) in the summer to less than 20 gpm in the winter of 1967-68.

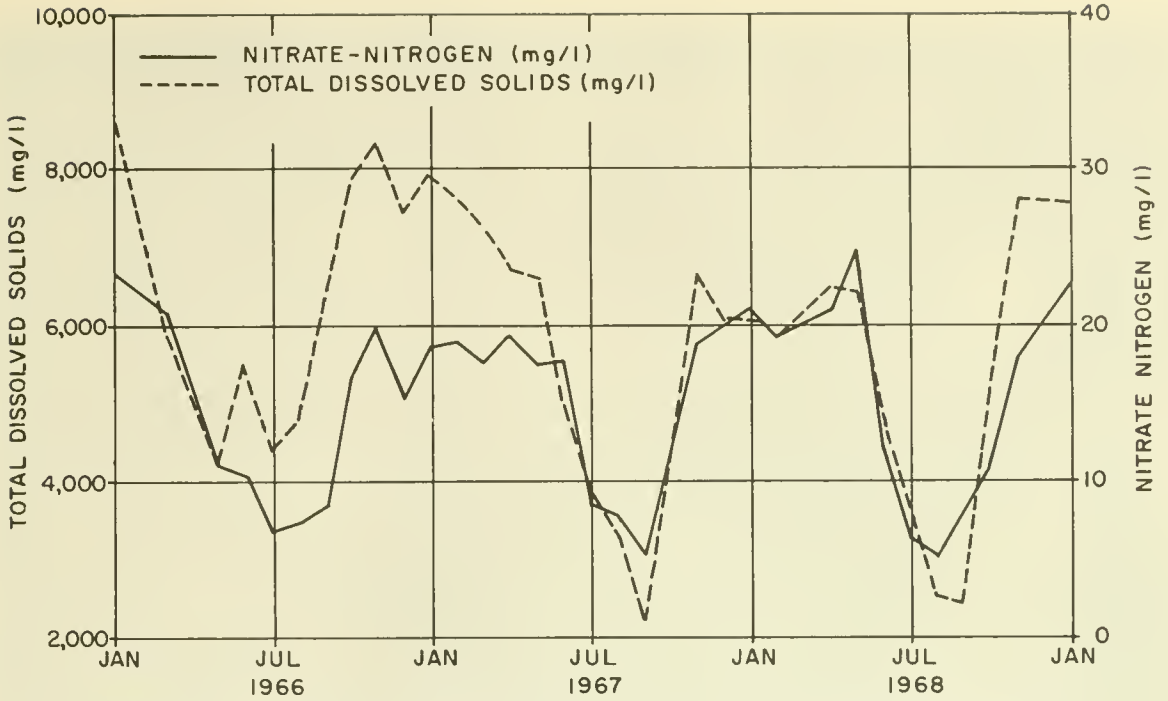


FIGURE 3 - SEASONAL VARIATION IN TOTAL DISSOLVED SOLIDS AND NITRATE-NITROGEN IN AGRICULTURAL DRAINAGE WATER AVAILABLE AT WASTEWATER TREATMENT CENTER

TABLE 3

CONCENTRATION OF MINERAL CONSTITUENTS IN TILE DRAIN WATER
(in milligrams per liter)

Constituent	Samples Taken at Alamitos Sump 5/2/67 (before flooding)	7/14/67 (after flooding)	Quantities Predicted for Master Drain, 1969
Calcium	373	194	220
Magnesium	190	106	160
Sodium	1,390	875	1,900
Potassium	5.4	4.1	20
Carbonate	0	0	0
Bicarbonate	247	373	220
Sulfate	3,600	2,070	3,500
Chloride	559	304	1,000
Nitrate	17.6	9	20
Hardness	1,710	921	1,200
Total Dissolved Solids	6,590	3,950	6,800
Boron	14	9.3	11

In Table 3 results of analyses made before and after flooding are compared with quantities predicted for the Master Drain. From these and other data, the Alamitos sump water appears to be quite similar to that of the predicted combined drain effluent during most of the year. However, flooding of the rice fields had a marked effect on water quality.

Analysis of the trace mineral results in Table 4 indicates that these constituents remained relatively constant throughout the year. Because of the semiquantitative nature of the emission spectrograph, probably no major differences (with the possible exception of molybdenum) are shown. These elements are probably of limited solubility; therefore, their concentrations may be independent of flow.

Because of the fluctuations of water quality illustrated in Figure 3, a covered storage pond was used to provide a constant supply of tile drainage water for the lightbox and all outdoor units. This pond, which could store about 820,000 gallons, was lined with polyethylene plastic to prevent loss of water or contamination by the ground water. The cover and lining minimized biological activity, thus providing a reasonably constant 30- to 90-day water supply depending on depth and detention time of the rapid growth pond.

FIGURE 4 - ALGAL GROWTH FACILITIES AT AGRICULTURAL WASTEWATER TREATMENT CENTER

TABLE 4

TRACE METAL ANALYSES OF ALAMITOS SUMP WATER DURING 1968-69

Element	Concentration - parts per billion Sample Date				
	12/10/68	2/12/69	3/20/69	4/8/69	9/6/69
Aluminum	31	1.4	2.5	1.7	14
Cadmium	23	20	2.5	12	11
Iron	8.6	4.3	3.2	10	6.3
Manganese	12	18	4.4	15	43
Molybdenum	40	17	32	77	57
Nickel	8.0	8.0	7.2	7.7	6.6
Vanadium	1.9	3.1	2.7	2.0	2.3
Zinc	5.7	5.7	10	6.7	5.7
Copper	2.9	1.4	2.5	1.7	1.7
Cobalt	1.4	1.4	2.5	1.7	1.4

Algal Growth

The effect of different variables was tested at three levels of culture size: lightbox batch assays, small 1,000-gallon outdoor growth units, and a large 1/4-acre demonstration pond. Because of lack of replicability of the large pond, most of the actual experimental work was conducted using the lightbox and small outdoor culture units. An aerial photograph of the Treatment Center (Figure 4) shows the location of the various facilities used in this investigation.

Lightbox

The lightbox was located in an air conditioned, converted office trailer provided with forced-air heating. The trailer was partially insulated and temperatures were

maintained at 25± 4°C. The lightbox consisted of two shelves, 12 x 3 feet, which held 200 one-liter culture flasks. No means of automated, mechanical agitation was provided, but air (compressed only or enriched with CO_2) could be introduced to individual flasks via a central manifold. Air volume was regulated by short sections of capillary tubing in each line and resulted in approximately equal amounts of air being delivered to each flask.

Four 12-foot cool-white fluorescent fixtures, comprising a total of eight lamps, were placed about 15 inches above each shelf. Ballasts were separated from the light fixtures to minimize local heating. Light intensities on the shelves ranged from 350 to 400 foot-candles, depending on location. The lighting was controlled by a timer to regulate the light-dark cycle.

Laboratory growth studies were customarily conducted with 1,000 milliliter (ml) erlenmeyer flasks containing 500 ml of culture. Each variable was tested in triplicate. Drainage water from the storage pond was used most of the time. On occasion, water was taken directly from the sump to determine whether storage affected the water's potential to support algal growth. A culture containing predominantly Scenedesmus from the outdoor growth units, usually the rapid growth pond, was used to inoculate individual flasks. The initial inoculum was normally on the order of 2,000 to 3,000 cells per milliliter. No attempt was made to obtain unialgal or bacteria-free cultures in these studies; however, the algae in the inoculum were normally 90 to 95 percent Scenedesmus.

Almost all lightbox studies conducted in 1969 used continuous lighting. No detrimental effect appeared, and the experimental time was shortened, allowing more assays to be run. In the laboratory studies, various levels of CO_2 enrichment were tried. The addition of CO_2 was accomplished by methods ranging from hand swirling, accelerated diffusion of atmospheric CO_2 into the culture medium, to injection of 100 percent CO_2. Temperature studies were conducted by immersing culture flasks to a one-inch depth in water baths at controlled temperatures.

Certain observations were made daily during the course of the studies. Because of the primary interest in nitrogen uptake, emphasis was placed on the collection of daily nitrate data obtained by means of the specific ion electrode. Other parameters that were often measured daily were pH, maximum and minimum temperatures, and fluorescence. At the end of a run, the triplicate flasks of each set of

variables were often pooled for volatile solids, total Kjeldahl nitrogen, nitrite, and cell count determinations. When the effect of various nutrient additions was tested, the stock solutions used were prepared from analytical or reagent grade chemicals.

Miniponds

A total of 22 small, resin-coated plywood ponds, termed "miniponds", were operated at Firebaugh. Eighteen of these ponds were 8 feet wide x 16 feet long with a designed operating depth of 12 inches (3 inches of freeboard). Approximate capacity of these ponds was 1,000 gallons. Two other ponds, also 8 x 16 feet, were used to study operating depths of 8 and 16 inches. The remaining two ponds (12 inches operating depth) were modified in the spring of 1969 to improve the hydraulic characteristics of the ponds, specifically to eliminate stagnant zones during mixing. A comparison of the two designs is shown in Figure 5.

All but two of the miniponds had mixing pumps whose capacity was approximately 80 gallons per minute (gpm). This provided average in-pond velocities of 0.25 to 0.5 feet per second (fps). Timers were placed in the electrical circuits of the pumps to vary mixing during a 24-hour period.

The flow of water from the storage pond was metered into ponds by means of individual flowmeters. All water lines were made of opaque material to prevent clogging by algal growth. Effluent was drawn from near the bottom of the storage pond and discharged through a "broken" siphon arrangement. This device was also used to maintain a constant water depth.

Normal operation of a minipond run began with the removal of all algae from a previous run. The ponds were then started with a common source of algae, usually from the RGP. For the first few days of a run, the ponds were operated either on a batch basis or at long detention times. This acclimatization period was designed to allow algal biomass to accumulate before being influenced by the experimental variables. Pond samples were collected daily from near the effluent at about 0830 hours and taken to the laboratory for analysis. In-pond pH values were determined once or twice daily by a portable pH meter.

All water delivered to the miniponds was taken from the storage pond and metered into the ponds by flowmeters (rotameters) that were adjusted daily. Periodically, the rotameters were calibrated by timing the flow of a known

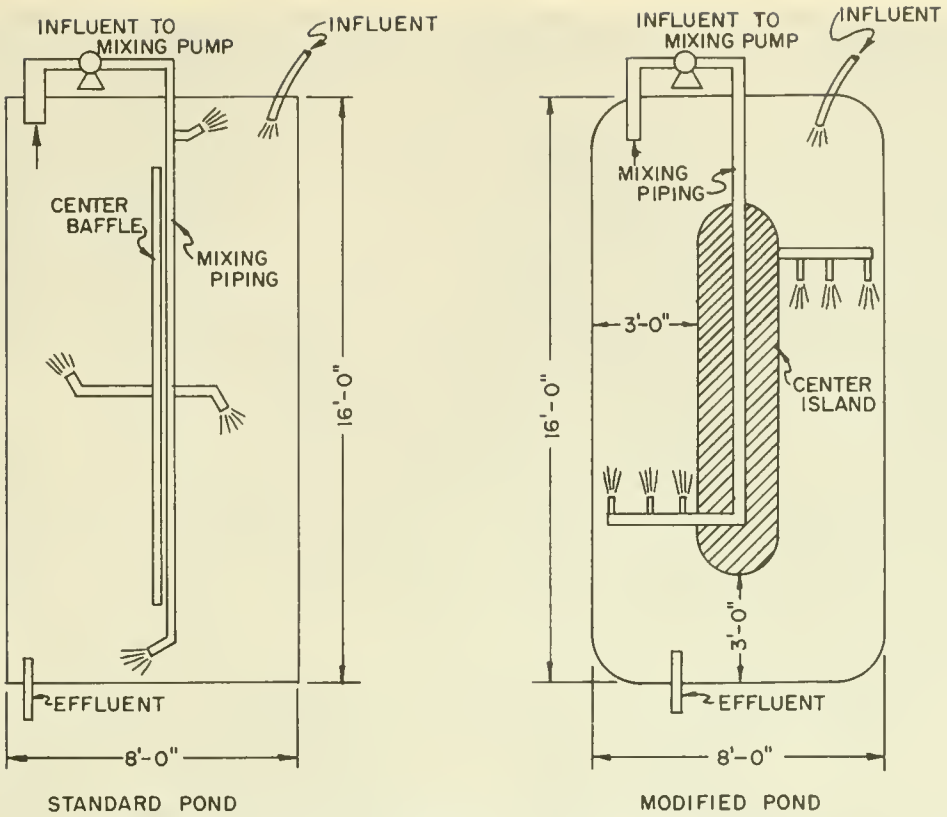


FIGURE 5- MINIPOND DESIGNS USED AT THE I. A.W.T. C.

volume and, if the meters were inaccurate, the glass tubes and stainless steel floats were cleaned in a potassium dichromate-sulfuric acid cleaning solution and then recalibrated. Nutrients were added to individual ponds or to the storage pond. Technical grade sodium nitrate was added to the storage pond to increase low summer nitrate concentrations to near 20 mgN/l. Often fertilizer grade phosphoric acid was added to the storage pond, but when phosphorus was being studied, it was added directly to the miniponds. The phosphorous concentration for most of the runs was 2 mg/l, as P. Iron ($FeCl_3$) was added daily to the growth units when tested as a growth factor.

Except during special mixing studies, the miniponds were mixed twice daily, from 0800 to 0830 hours (for sampling) and from 1200 to 1530 hours. During most of the study, the miniponds were also swept once daily to eliminate concentrated algal deposits caused by eddy currents. Carbon dioxide (5 or 100 percent) and atmospheric air were injected

into some ponds through the intake side of the mixing pumps during the afternoon mixing cycle only. Bottled CO_2 was mixed with compressed air to obtain the desired concentration of carbon dioxide. The true amount of CO_2 injected was estimated by recording changes in cylinder weight.

During some minipond runs in 1969, a settling tank consisting of a 50-gallon drum with an influent line entering near the center of the drum was attached to one of the miniponds. Influent water came from one of the nozzles of the mixing system and a surface overflow returned the water to the pond. The algal sludge was periodically removed by draining from an opening near the bottom of the tank.

During 1968, Sacramento blackfish (Orthodon microlepidotus) were added to some miniponds to test the possible effect of fish on algal growth. The fish ponds with mixing had a screen placed over the intake side of the mixing pump to prevent the fish from being brought in the pump suction.

The California Department of Fish and Game used two miniponds to determine whether Clostridium botulinum might grow in this type of aquatic environment. One pond received a one-inch layer of soil from an area with a known botulism outbreak (Tulare Lake), while the remaining pond received one inch of soil from the Treatment Center site. Personnel from the Department of Fish and Game made periodic visits to the Center to sample the two ponds for bacterial analyses. These two ponds were also routinely sampled with the other miniponds; however, the only controlled variables were water depth (12 inches), P addition (2 mg/l), and detention time. The ponds were neither emptied, mixed, nor swept during the study period.

Rapid Growth Pond (RGP)

The rapid growth pond (Figure 6) is an asphalt-lined pond with a 12.5-foot wide folded raceway approximately 800 feet long. The center baffles were originally constructed of sheets of aluminum attached to both sides of a wooden, upright frame; however, algae accumulated between the two sheets and one sheet was removed. The RGP could be operated at depths from 0.5 to 3 feet, with the effluent taken from either the top or bottom of the pond. With its four available mixing pumps, velocities of 1 fps were theoretically possible at any pond depth. This mixing velocity was considered necessary to achieve complete resuspension of settled algae and periodic mixing and aeration of the bottom sludge layer. Each pump had an individual timer which allowed an almost infinite variety of mixing schedules.

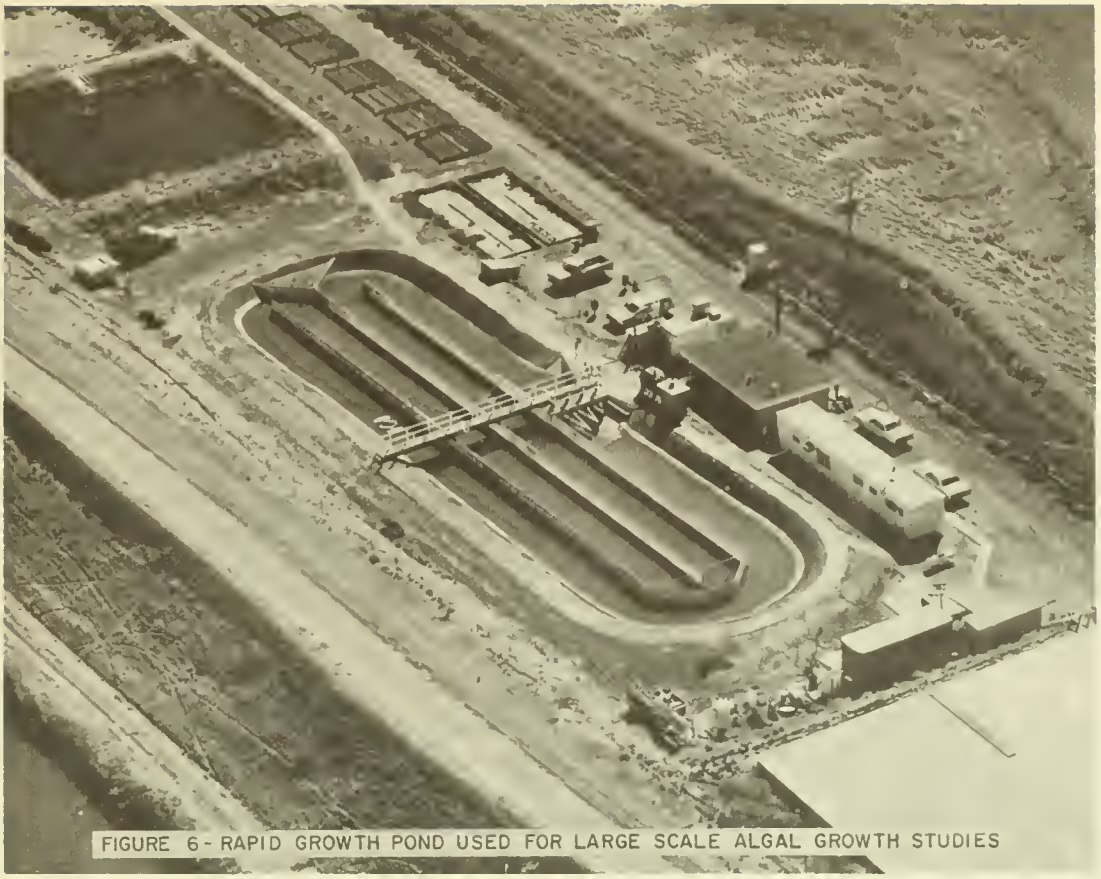


FIGURE 6 - RAPID GROWTH POND USED FOR LARGE SCALE ALGAL GROWTH STUDIES

Influent from the storage pond was pumped into the RGP semicontinuously throughout a 24-hour period. Detention time was regulated by varying the length of time the influent pump functioned during each specified timing interval and by the flow of the pump. The effluent pump, also on a timer, could be programmed to pump at a specific time or as a function of pond depth. Recording flowmeters were attached to both influent and effluent pumps to record daily the amount of water entering and leaving the pond. A recirculation pump was available to introduce the pond culture into the influent line.

Operation of this growth unit resembled that of the miniponds. The optimum levels of experimental variables noted in minipond runs were maintained as nearly as possible in the RGP. Because of its uniqueness, the pond was used mainly as a demonstration unit and as a source of algae for separation studies. During the 1969 operation of the RGP, daily changes often were necessary in mixing, sweeping, and so forth, to provide algae for testing various pieces of separation equipment.

Algal Harvesting

Laboratory

The laboratory used in the harvesting studies was separate from the chemical and biological laboratories described previously. Standarized coagulation-flocculation tests ("jar tests") were conducted in this laboratory to screen potential coagulants in the concentration step of algal harvesting. The coagulants tested included such compounds as lime ($Ca(OH)_2$), alum ($Al_2(SO_4)3$) and ferric sulfate ($Fe_2(SO_4)3$), as well as various polyelectrolytes. These tests were used to estimate the effectiveness of these different compounds for coagulation-flocculation before testing in the larger, pilot-scale units. Routine tests of the most promising test compounds, or combinations of compounds, were also conducted over extended periods of time to determine possible seasonal variations in their effectiveness. The experimental procedures used for these tests are outlined in the following paragraphs.

Suspensions of algae for the jar studies were obtained directly from outdoor growth units, usually the rapid growth pond. The suspensions had volatile solids concentrations of from 50 to 800 mg/l. In making the daily comparisons of various chemical coagulants, a common supply of algae was used. It was collected on the morning of the testing period. The procedure for the jar tests was standardized as follows: 800 milliliters of algae suspension were placed in 1,000 ml "tall form" beakers and the calculated quantity of reagent was added. The samples were then mixed by means of a multimixer at a specified paddle speed and time, depending on the type of coagulant used. The speed and duration were 40 rpm for eight minutes with lime and 70 rpm for three minutes with alum. These values were found to be optimum for maximum flocculation. If polyelectrolytes were used in conjunction with alum or lime, the conditions optimum for the principal chemical were used. If polyelectrolytes were tested alone, the mixing speed and duration most effective for alum were employed, although mixing speed and duration were optimized for the most promising polyelectrolytes. After the mixing operation, the beakers were placed in a darkened area of minimum air circulation. At the end of one hour, the sample supernatant was decanted and a spectrophotometer was used to determine the percent transmittance of the supernatant at 410 mμ.

During the first part of these laboratory studies, the amount of algal biomass (and other suspended solids) removed by coagulation-sedimentation was determined by using a few random samples to establish the relationship between

weight of biomass in the flocculated material and percent transmittance (at 410 mμ) of the supernatant. A plot of these values was then used to estimate removal in the remaining samples. This method proved to be tedious and it was modified in the following manner. The suspended solids and percent transmittance were determined for a sample of algae-laden water from a growth unit. The sample was acidified with two drops of 6N H_2SO_4 to remove precipitated calcium carbonate. Several quantitative dilutions were then made of the original pond sample and the percent transmittance measured for each of the acidified dilutions. The calculated suspended solids versus the recorded transmittance for each dilution were then plotted. A typical curve is shown in Figure 7. The percent transmittances of the acidified supernatant from the flocculated samples were then determined, and the removal percentage or total suspended solids remaining were then obtained from the dilution curve.

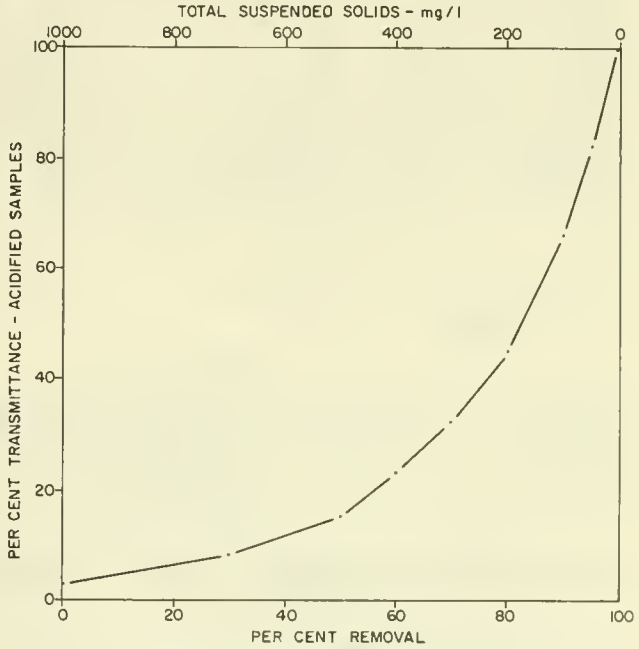


FIGURE 7 - TYPICAL DILUTION CURVE USED TO DETERMINE TOTAL SOLIDS FROM PER CENT TRANSMITTANCE

Chemical analyses occasionally performed on the supernatant included nitrate, nitrite, organic nitrogen, orthophosphorus, pH, hardness, calcium, and magnesium. All analyses were performed as outlined in the section on growth, except that calcium and total hardness were determined by the EDTA (ethylenediaminetetraacetic acid) titrametric method, with magnesium calculated as the difference between the two ions.

Pilot-Scale Separation Studies

The equipment used in these studies, which reflected the types readily available for lease or rental, dictated the testing procedures that were followed. (Nothing in the following discussion is intended as an endorsement of any type of equipment.) Descriptions of the units and testing procedures are contained in the following paragraphs.

Sedimentation Tank. A rectangular sedimentation tank was borrowed from the Los Angeles County Sanitation District and was used in the removal of algae and sediment from the rapid growth pond. The unit was approximately 4 feet wide x 6 feet deep x 22 feet long with a capacity of about 4,000 gallons. Water was pumped from the RGP to the bottom of the tank and effluent was withdrawn from near the top and returned to the growth unit. The settled algal slurry was moved to one end of the tank by blades attached to a continuous chain and was periodically discharged onto drying trays. Detention time was the only controllable variable.

Shallow Depth Sedimentation Tank. This was a self-contained water treatment plant called the "Water Boy" obtained from Neptune Microfloc, Corvallis, Oregon. The Water Boy was the only unit consistently operated on-line with the RGP. The plant treated a portion of the pond effluent and discharged its waste from the site. A schematic flow diagram of the unit is shown in Figure 8. As illustrated in the diagram, the unit consisted of a flocculation chamber, a settling chamber, and a mixed-media filter.

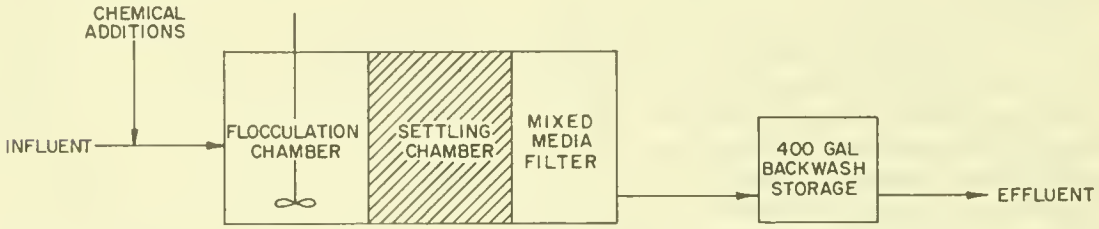


FIGURE 8 - SCHEMATIC AND FLOW DIAGRAM OF WATER BOY SHALLOW DEPTH SEDIMENTATION UNIT

In the flocculation chamber the algae-laden water was mixed with a coagulant (or coagulants) in concentrations determined by jar test studies, and from there passed to the settling chamber. The chamber differed from the one described in the preceding paragraph in that it was equipped with a module of settling tubes inclined at an angle of $7\frac{1}{2}°$ upwards in the direction of flow. The theory behind this type of sedimentation unit has been described by Hansen, et al (1969). Essentially, the settling tubes reduce particle settling distances and thus lower the time required for sedimentation (i.e., detention time). Figure 9 illustrates the effect of tube inclination on settling distances of discrete particles. (Diagram and following description are taken from Hansen, et al, 1969).

"The path traced by a particle settling in a tube is the resultant of two vectors: V, the velocity of flow through the tube, and v_s, the settling velocity of the particle. It can be seen in Figure 9 that if the settling surfaces are inclined upward in the direction of flow, the settling path of the particle is altered because the component of the settling velocity which is parallel to the tube wall, v_{sh}, is opposite in direction to the velocity vector V. If V is greater than v_s, the required length of the settling surface decreases as the angle increases from zero up to about 25 to 30 deg. (at $V = 2.5\ v_s$) and then increases, approaching infinity as the angle of inclination is increased to 90 deg. For $V < v_s$ the tray length continues to decrease with increasing angle."

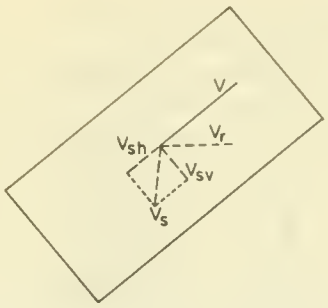


FIGURE 9 - EFFECT OF TUBE ANGLE ON SETTLING PATH OF SUSPENDED PARTICLE

Use of the tubes also reduces turbulence and short-circuiting, thus promoting laminar flow of the water. With periodic flow reversal, or drainage, the inclination of the tubes facilitates removal of the algal floc. The coagulation-sedimentation step was designed to remove most of the suspended solids; however, a mixed-media filter provided a final polishing of the effluent. After passing the filter, most of the clarified water went to discharge with only a small portion being stored in the backwash tank for backwashing the filter. The settled algal suspension from this unit normally was discharged into a holding sump to provide material for study of dewatering equipment.

The Water Boy was used only to remove algae from growth pond water, that is, the concentration step. Controllable variables included hydraulic detention time, type and amount of flocculate, and frequency of sludge removal. The unit, as tested at the IAWTC, required manual operation of the filter backwash and sludge removal cycles.

Upflow Clarifier. In the upflow clarifier constructed at the site (Figure 10) a coagulating aid, usually sodium hydroxide, was added to the influent algae-laden water from the RGP. The mixture then entered the apex of an

inverted cone and flowed through a thin diffuser layer to mix the coagulant and algae-laden liquid. The algal floc flowed upward and, as the cross-sectional area increased, the velocity decreased, causing the floc to settle. A mat of algal floc formed at the point at which the upward velocity of flow equalled the settling velocity of the floc. The algal sludge was drawn off from the mat, and the clarified liquid passed off at an upper level. Operational variables were detention time and concentration of coagulant.

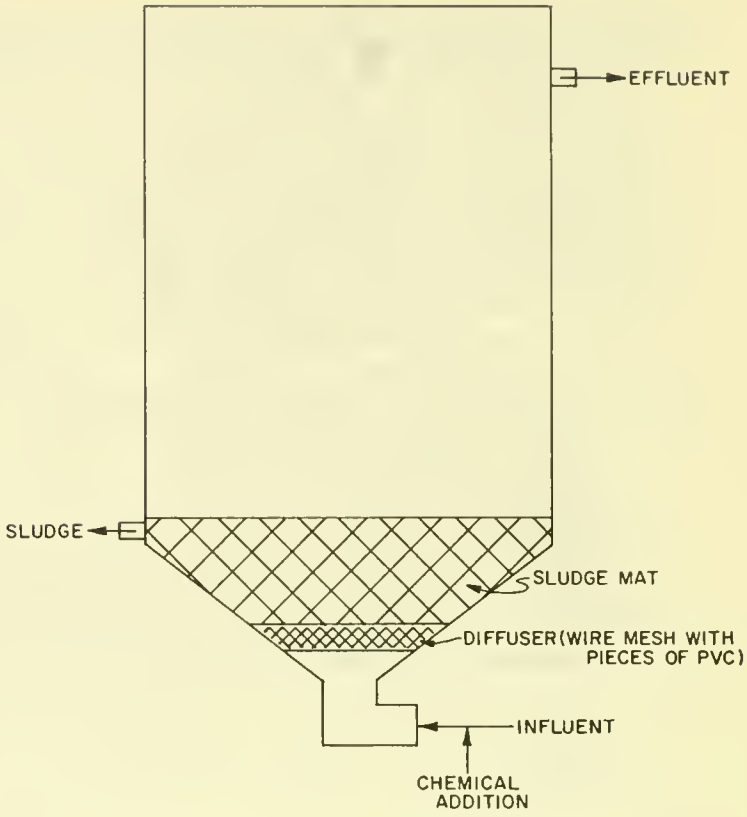


FIGURE 10-SCHEMATIC DIAGRAM OF UPFLOW CLARIFIER

Upflow Sand Filter. A Sanborn filter was constructed for the algae stripping project by Bohna Engineering and Research, Inc., of San Francisco, California. The principle of operation for this unit is illustrated in Figure 11. A description of the principle is quoted from an unpublished Bohna internal report dated February 6, 1970:

> "The fluid to be filtered is pumped into a feed chamber at the base of the filter from where it flows upward in a thin channel called the feed wick which is closed at the top. The fluid then flows horizontally across a polypropylene cloth surface followed by a thin layer of sand. A second layer of cloth backs up the sand and allows the clarified liquid to enter a second channel called the drain wick from where the filtrate flows upward into a backwash-holding tank and then to final disposition.

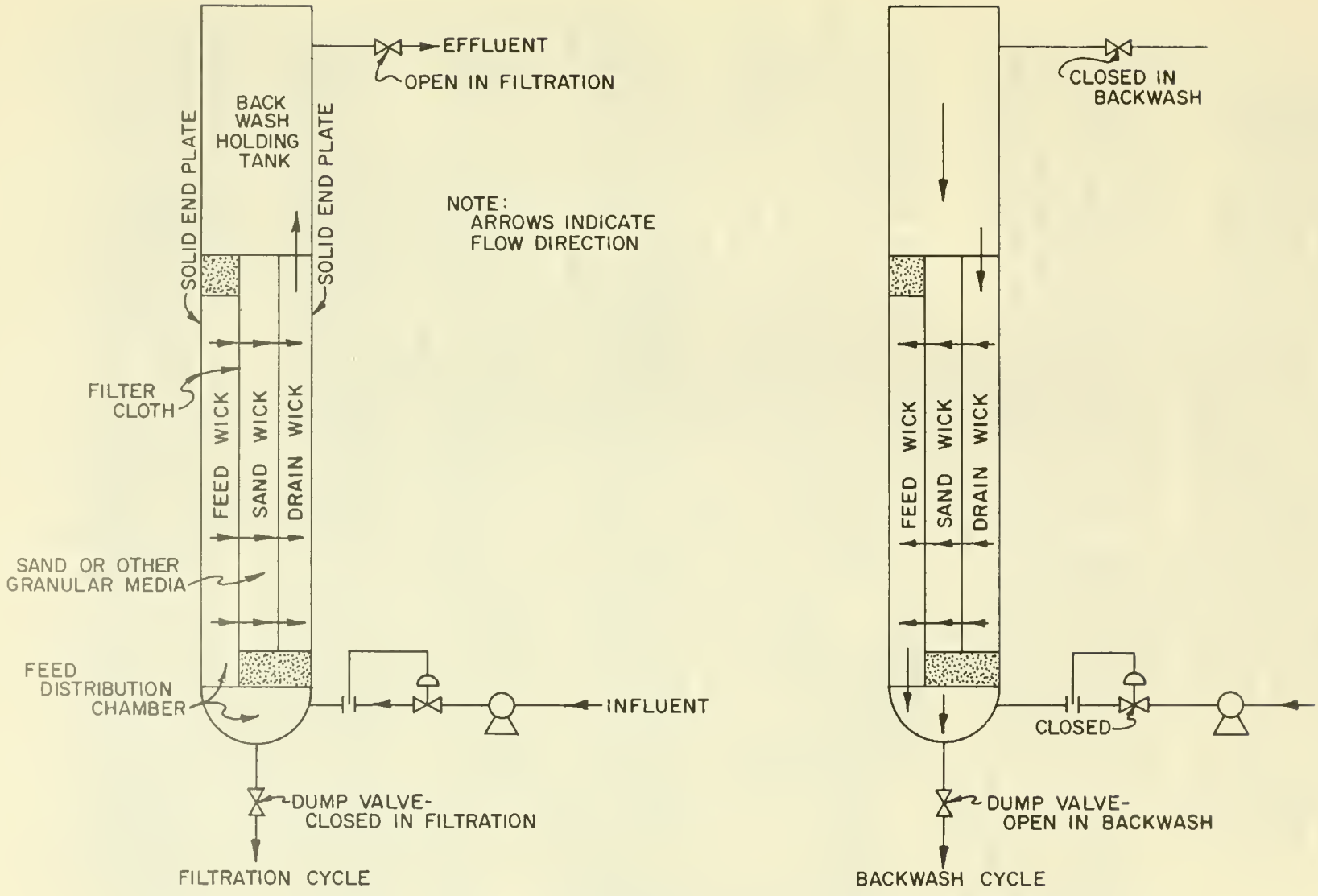


FIGURE 11 - SCHEMATIC DIAGRAM AND PRINCIPLE OF OPERATION OF SANBORN FILTER

"In backwashing, the overflow effluent valve and feed valves are closed and a dump valve at the base of the feed chamber is opened instantaneously, allowing the hydraulic head to force filtered water down the drain wick, through the sand and out the dump valve, thereby removing the accumulated solids from the filter cloth and sand bed."

This filter was operated on water directly from the rapid growth pond, without any chemical pretreatment, and the filtered effluent was discharged from the site. Most of the data for this unit were obtained during two intensive studies with supervisory help from Bohna. Controllable variables included influent flow rate, backwash volume, and the pressure differential across the filter media at which the unit was backwashed.

Microscreen. An automatic rotating drum filter leased from the Zurn Company consisted of a 2-foot wide, rotating drum having a diameter of 4 feet and mounted in a self-contained unit. Algal-laden water entered the interior of the partly submerged drum and was filtered as the water flowed outwards through a screen attached to the revolving drum. Screens of 25 and 35 micron mesh size were supplied with the unit. Algae caught on the screen were washed off by a row of water jets located above the drum; and the algal slurry then dropped into a trough located inside the drum, above the water level. The microscreen was tested primarily as a concentrating device, but also as a means to dewater an algal slurry from the Water Boy. The major variable tested was the amount of solids loading per unit area of screen.

Vacuum Filter. A continuous belt vacuum filter was leased from the Eimco Corporation to examine its feasibility both as a concentrating and as a dewatering device. A schematic diagram of the unit is shown in Figure 12. The filter media, a continuous porous belt, was immersed in the filterable material. A vacuum applied on the inside of the drum caused the suspended particles to form a cake on the belt. As the belt passed over the discharge roll, a cake deflector scraped off the cake. The belt was then washed as it passed back into the feed tank.

The influent algal suspension for this unit contained from 0.3 to 3 percent solids. The number of possible experimental variables was quite large and included flow, algal concentration, filter material, drum speed, and amount of vacuum. With the exception of drum speed, all these variables were tested at the IAWTC.

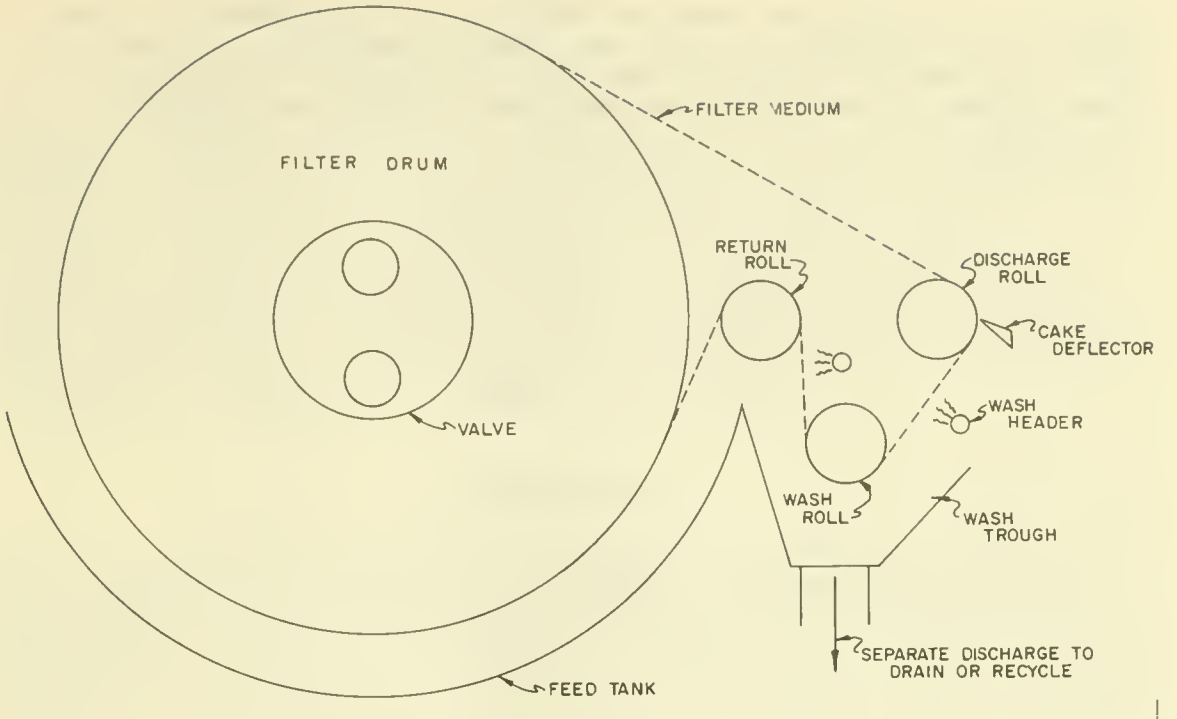


FIGURE 12 - PRINCIPLE OF THE BELT FILTER

Centrifugation. Three types of centrifuges were investigated as possible means of primary concentration and/or dewatering. The first of these units was a Bird Machine Company continuous flow, solid bowl centrifuge consisting of two concentric rotating elements inside a stationary housing. The inner element was a scroll-like unit rotating at a slightly higher speed than the outer element. Variables tested, using both straight pond water and a concentrated slurry, included flow, centrifuge speed, and liquid depth in the bowl.

The second variety of centrifuge was a De Laval yeast-type separator built by the De Laval Separator Company which was designed for continuous feed and removal with solid/liquid and solid/liquid/liquid extraction. Centrifugal force in this type of unit is constant and discharge of solids is controlled by varying the size and number of nozzles. The third centrifuge was a De Laval self-cleaning continuous flow unit. In the last-named type of centrifuge, suspended solids accumulate on the side of the bowl until they reach a predetermined level and then are discharged as the bowl opens. Once the solids have been ejected, which occurs in only a matter of seconds, the bowl closes and normal operation continues. During opening and closing of

the bowl, bowl speed remains unchanged. Experimental variables in testing this unit were feed composition (used as both a concentration and dewatering device), throughput (flow), length of time the bowl was open, and amount of bowl discharge.

CHAPTER IV - RESULTS AND DISCUSSION

Data are presented in this section which were obtained in algal growth and nitrogen assimilation and harvesting studies at the Treatment Center. Also included are the data of two other studies which accompanied algal growth -- regrowth and botulism. The regrowth studies were concerned with the effectiveness of treatment methods for removal of those substances from agricultural wastewater which promote or "stimulate" algal growth. In the botulism work, the research was designed to evaluate the possibility of botulism outbreaks in algal growth units.

Algal Growth

The investigation of algal growth was divided into three levels of interest -- lightbox, miniponds, and rapid growth pond. The major emphasis in these studies was to determine the effect of various factors on the rate at which nitrogen is assimilated by algae in laboratory and outdoor cultures. To achieve continuity of results in these studies, nutritional and physical factors were made most favorable for the growth of Scenedesmus. It should be pointed out that there are other algae which are probably equally suitable for nitrogen removal. The last part of this section on algal growth will provide some general information on various biological, chemical, and physical factors recorded during the course of the investigation.

In this section many of the results will be reported in terms of nitrogen assimilation, expressed either as mg/l or percent. In general, "nitrogen assimilation" is expressed or measured as the amount of soluble nitrogen disappearing (i.e., removed) from a medium as a result of algal growth. This method of expressing nitrogen assimilation is a simplification of the true system found in algal cultures where growth and decomposition are occurring simultaneously and a constant flux of nitrogen forms takes place. For example, in most cultures dissolved organic nitrogen increased with culture age, probably from extracellular excretion or cellular decomposition. As defined in this report, this would be considered to be nonassimilated nitrogen. Nitrogen balances obtained by determinations of both influent and effluent values indicated that nitrogen loss through anaerobic denitrification and release as nitrogen gas was not significant.

Lightbox Studies

Laboratory cultures of Scenedesmus were used to determine optimum nutritional requirements of the algae, the effect of temperature on algal growth, possible effects of seasonal variation in mineral quality of the tile drainage, and comparisons of algal growth and nitrogen assimilation in tile systems other than the system used at the Center. In addition to these studies, the lightbox was used to evaluate the growth rate and nitrate uptake in tile drainage of algal species other than Scenedesmus. Because of the large numbers of flasks involved in the lightbox culture studies, nitrate was the only nitrogen form determined routinely. Occasional analyses for other forms indicated that nitrite and ammonia usually were present in amounts less than 0.10 mg/l and organic nitrogen in amounts ranging from 0.5 to 0.8 mg/l. Changes in nitrate were thus valid indicators of the effect of a variable on total nitrogen assimilation. The results of the batch culture studies were then used in outdoor cultures to provide optimum growth conditions for the algae. The results of these studies are detailed in the following paragraphs.

Nutrient Requirements. Batch assays were first used to determine the necessary nutrient additions for optimum algal growth and nitrogen assimilation in the tile drainage. Because the ratio of nitrogen to phosphorus (P) in Alamitos sump water is about 100:1 (as compared to approximately 10:1 in the algal cell), phosphorus was the first element tested. Figure 13 illustrates results of a typical experiment which compares 0 and 2 mg/l P addition to Scenedesmus cultures. In this particular study, unconverted nitrate meter readings, which are proportional to nitrate concentrations, were plotted instead of actual nitrate values. The meter readings were often used with the assumption that relative changes were a reliable indication of a variable's effect. At the end of five days, the culture to which no phosphorus was added, remained at, or near, the initial nitrate level; whereas, the culture containing 2 mg/l additional phosphorus showed a 50 percent reduction in dissolved nitrate.

In general, all lightbox studies indicated that little or no growth occurred without supplemental phosphorus. The 0.2 mg/l originally present in the tile drainage water was not adequate for attaining the biomass levels of Scenedesmus cultures required for maximum nitrogen assimilation. All subsequent laboratory cultures received 2 mg/l PO_4-P, unless phosphorus was one of the experimental variables.

After the necessity for adding phosphorus had been established, further experiments were conducted on the effect of additional iron on Scenedesmus cultures. A typical example of this type of assay is shown in Figure 14 where $FeCl_3$ was used as the source of iron and the lighting cycle was set for 12 hours light and 12 hours darkness. The combination of 2 mg/l P plus 3 mg/l Fe led to an increase in the rate and amount of nitrate assimilation. Experiments with different concentrations of Fe indicated that 3 mg/l (from $FeCl_3$) produced nearly optimum results. Two additional iron compounds, ferrous sulfate ($FeSO_4$) and ferric citrate ($FeC_6H_5O_7$) were tested. The results of the test showed that while both were adequate sources of Fe, optimum concentrations differed. With the use of $FeSO_4$, the requisite concentration was similar to that of $FeCl_3$, or 3 mg/l Fe. However, when ferric citrate was used, about twice as much iron (as Fe) had to be added in order to achieve the same rate of nitrate assimilation. The use of a chelating agent, the sodium salt of EDTA, decreased the concentration of iron required. For example, in one study 3.2 mg/l Fe alone was as effective as 0.4 mg/l Fe combined with 5.0 mg/l EDTA addition. EDTA alone also resulted in an increase in nitrate assimilation, presumably by making the small amount of iron in the sump water available for algal growth. Cost analyses of the use of EDTA in large-scale algal cultures indicated that the use of this

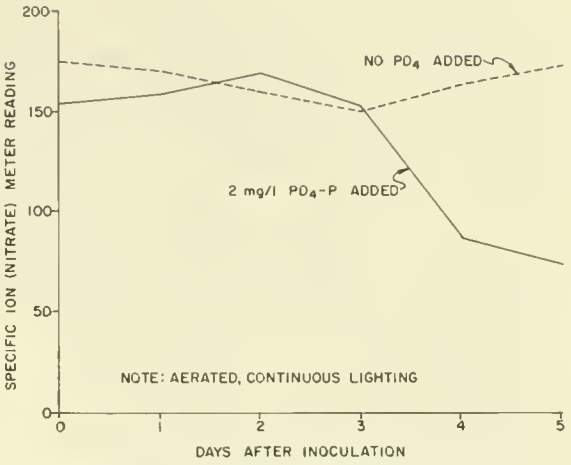


FIGURE 13 - EFFECT OF PHOSPHOROUS ADDITION ON NITROGEN ASSIMILATION

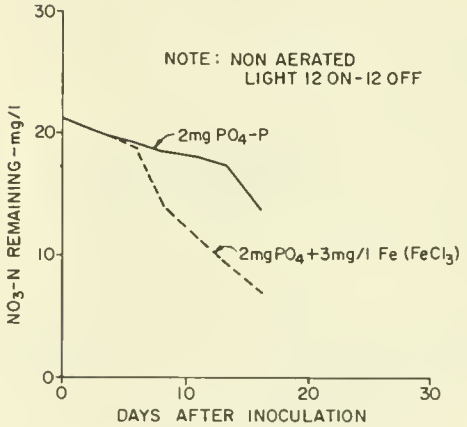


FIGURE 14 - EFFECT OF IRON ADDITION ON NITROGEN ASSIMILATION BY SCENEDESMUS CULTURES

compound was not economically practical. After several replications of the iron studies, 3 mg/l of Fe (from $FeCl_3$) was routinely added to lightbox algal cultures.

Based on theoretical considerations, Alamitos sump water did not contain enough carbon to support the growth of biomass required to assimilate 20 mg/l nitrate-nitrogen. This deficiency indicated that CO_2 addition to laboratory cultures could enhance nitrogen assimilation by Scenedesmus. Studies were then initiated using a mixture of 4 percent CO_2 in compressed air, compresed air only, and surface reaeration (flasks hand-swirled twice daily). The results of one of the experiments are illustrated in Figure 15, which shows that the rate of nitrate uptake was significantly increased by the addition of of 4 percent CO_2, and that compressed air also was an effective means of providing some additional carbon. The pH values were about 7.2 to 7.3 for the 4 percent CO_2, 9.6 to 9.8 for the compressed air, and 10.0 to 10.5 for the swirled cultures. On two occasions, various amounts of sodium bicarbonate were added to inoculated sump water to explore the possibility of using this compound as a carbon source of nitrogen assimilation. These cultures were then compared to other cultures receiving 4 percent CO_2. In both of these studies, the addition of bicarbonate did not enhance nitrogen assimilation.

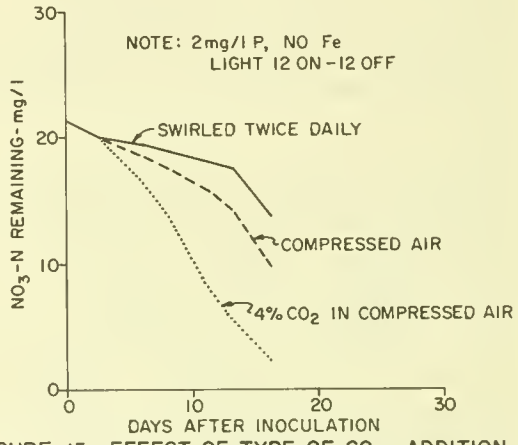


FIGURE 15 - EFFECT OF TYPE OF CO_2 ADDITION ON NITROGEN ASSIMILATION BY SCENEDESMUS CULTURES

Although 4 percent CO_2 was conclusively shown to benefit algal growth in the cultures, in almost all of the subsequent experiments at least two levels of aeration were applied, namely injection of 4 percent CO_2 and diffusion of atmospheric CO_2 by swirling the flasks twice daily. The two levels of CO_2 addition were used to make the data more applicable to outdoor cultures, some of which received additional inorganic carbon.

The three levels of nutrients -- Fe, P, and CO_2 -- were then combined in a factorially designed experiment to determine their effect on nitrogen uptake by Scenedesmus cultures. Five levels of Fe at three levels of P were compared in flasks that received either 4 percent CO_2 or

compressed air. In addition, five levels of Fe were tested in cultures to which 2.0 mg/l P had been added and which were swirled twice daily. The nitrate remaining in the various cultures is shown in Figure 16. These data indicate that interaction occurred between the three variables tested, and that in general, the addition of CO_2 and Fe increased the rate of nitrate assimilation. The level of iron addition which provided the maximum effect was about 3 mg/l of Fe. The minimum requirement for complete nitrate assimilation at 11 days was 4 percent CO_2, 2 mg/l P, and 3 mg/l Fe. The addition of higher levels of Fe and P probably resulted in

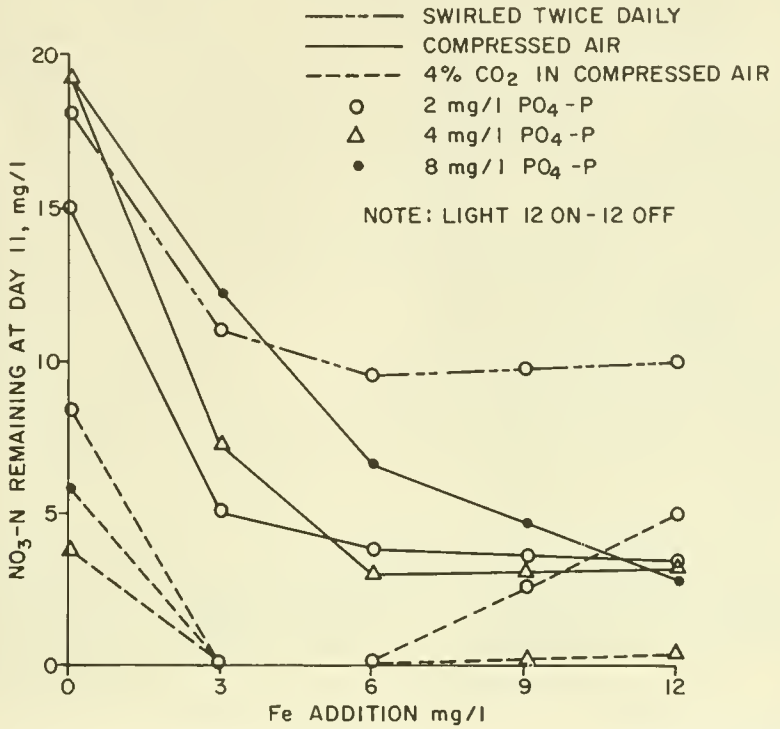


FIGURE 16 - NITRATE-NITROGEN REMAINING AT DAY 11 IN SCENEDESMUS CULTURES WITH VARIOUS COMBINATIONS OF Fe, P, AND CARBON DIOXIDE

the formation of an unavailable iron-phosphate complex. By day 16, all aerated cultures (with and without CO_2) receiving the addition of 3 mg/l or more Fe had reached zero nitrate concentration.

Carbon dioxide undoubtedly played a dual role in these culture studies by supplying additional inorganic carbon and lowering the pH to such an extent that many elements that ordinarily precipitated in algal cultures remained in solution. In most of the laboratory culture work at Firebaugh, 4 percent bottled CO_2 was used; however, on occasion 100 percent CO_2 was tested. The addition of

100 percent CO_2 was never found to be more effective than 4 percent CO_2 and often was detrimental to algal growth, perhaps because pH values were less than 6. This value falls below the optimum range of 7 to 8.5 for Scenedesmus. Oswald (1963) found that about 0.5 percent CO_2 addition to mixed cultures of Chlorella and Scenedesmus was most favorable for maximum light conversion efficiency.

Possible seasonal variations in iron and phosphorous requirements resulting from changes in the influent water quality were checked by conducting periodic laboratory studies in which cultures received various concentrations of these elements. These tests were normally run when the storage pond was being refilled. In all but one of these studies, 2 to 4 mg/l of both elements were necessary for maximum nitrogen assimilation. In the one exception iron was not required. Also included in many of these checks were comparisons of nitrate assimilation rates in sump water and storage pond water. Little or no difference was shown between the two waters, provided that both had comparable amounts of N, P, and Fe. In conducting this type of study throughout the year, seasonal differences were noted in nitrogen assimilation rates in those cultures receiving the optimum nutrient additions. Although these differences may be attributable to variations in inocula, they could have been the result of changes in water quality. This possibility will be discussed more fully in the section on comparison of water from various tile systems.

On several occasions trace minerals were added to the cultures to determine their effect on algal growth. These elements seldom had any significant effect on nitrogen uptake, either individually or in commonly used trace element solutions. The few instances in which a beneficial effect was noted, the results could not be duplicated in further experiments.

Temperature. A preliminary study on the effect of temperature on nitrogen uptake indicated that, of the three temperatures first tested (10°C, 20°C, and 30°C), 20°C was optimum for nitrate assimilation, and that Fe and P were necessary at all temperature levels. At 30°C, the initial Scenedesmus culture was replaced by a blue-green alga, Oscillatoria. The 10°C cultures had an extremely long lag phase and did not enter the exponential growth phase until near the end of the study. Therefore, another experiment was designed in which temperatures of about 15°C, 20°C, and

30°C were used. The apparatus used to maintain the various temperatures did not permit testing the effect of 10°C at the time of the test. This second study differed from the first in two important respects -- (1) three levels of CO_2 addition (none, 4 and 100 percent) were tested at all temperatures and (2) all of the cultures were allowed to grow at 20°C for four days before being subjected to the various experimental temperatures. (In the preliminary study, all culture flasks had reached the experimental temperature when they were inoculated.) When the cultures had assimilated all the nitrate in the flask, an additional 20 mg/l of nitrate-nitrogen was added along with 2 mg/l P and 3 mg/l Fe. Growth of Scenedesmus at the high temperatures followed the same pattern as that observed in the previous study -- rapid initial growth, then dying away, with eventual replacement by Oscillatoria. This species change was noted when either no CO_2, or 4 percent CO_2 was added but not with 100 percent CO_2. The use of pure CO_2 at all temperatures gave the same result: namely, a pale culture of apparently viable Scenedesmus which did not grow. The lack of growth may have been due to the low pH levels of these cultures (5.7 to 5.9). The 15°C cultures exhibited a surprisingly high rate of nitrogen uptake, especially with 4 percent CO_2 addition. Table 5 summarizes the total nitrate assimilated by the different cultures. With the exception of the 100 percent CO_2 cultures, the only combination of variables that produced a notable effect was the 4 percent CO_2 concentration at a temperature of 15°C. The inoculum used in this study was obtained from outdoor growth units which had been at a temperature of about 15°C for several days. Perhaps the period of acclimatization in the laboratory was not long enough for complete adjustment to the experimental temperatures.

TABLE 5

TOTAL MILLIGRAMS NITROGEN ASSIMILATED BY ALGAL CULTURES AT DIFFERENT TEMPERATURES AND CO_2 ADDITIONS

Temp. °C	Percent CO_2 Added		
	0	4	100
15	11	32	1
20	16	17	3
30	11	11	1

Algal Species. During the spring of 1969, unialgal cultures of 30 species of algae were obtained from the culture collection of the University of California at Davis. These algae were then cultured in drainage water, with and without P and Fe additions, and their rates of nitrate uptake compared to that of Scenedesmus from the outdoor growth unit. Of the species tested (including green, blue-green, and diatoms), six grew well in sump water plus nutrients, and one grew in the P-deficient medium. The six species included three green algae -- Ankistrodesmus, Eucapsis, and Gleocapsis; and three blue-green algae -- Anacystis, Oscillatoria, and Anabaena. By far the best growth and nitrogen assimilation were noted in Ankistrodesmus and Anacystis cultures; however, growth and nitrogen assimilation were not appreciably better than those of Scenedesmus. With no apparent advantage to be gained from using these algae, no concerted effort was made to develop large-scale cultures of the species tested.

Water Source. In a study of this type, where the water comes from an isolated system, one of the questions raised concerns the applicability of the data to an area-wide system. Although such combined drainage was not available during the project, samples of other tile systems were collected and tested for their algal stimulatory potential. As the partial chemical analyses of the samples shown in Table 6 indicate, the sumps differed considerably in nitrate and electrical conductivity (EC). The differences in nitrogen concentration were nullified by bringing all sumps to about 55 mgN/l as nitrate. Part of this study was performed to determine whether or not iron and phosphorous additions were needed to produce the algal biomass required to assimilate the nitrogen in each of these waters. The results showed that both elements were necessary and that there was

TABLE 6

CHEMICAL ANALYSES OF VARIOUS SUMPS

Sump	umho/cm^2	Concentration - mg/l				
		Total Kjeldahl	NO_3-N	NO_2-N	PO_4-P	Total Fe
DPS 1367	5890	0.28	45	0.003	0.06	0.03
HMV 7016	3880	0.30	55	0.003	0.00	0.04
GSY 0855	8100	0.25	16	0.004	0.00	0.07
BVS 7402 (Alamitos)	5200	0.40	13	0.006	0.038	0.05

little or no algal growth without their addition to the media. The curves in Figure 17 show changes in specific ion meter readings with time for two of the sumps: GSY 0855 and BVS 7402 (Alamitos). Data from HMH 7016 and DPS 1367 were similar to those of Alamitos and were omitted from the graph for reasons of ease of reading. The curves illustrate definite differences in the rate of nitrate uptake, with the algae in GSY 0855 water removing all the original nitrogen in about 7 days. The algae grown in the other drainage water required about 12 days to remove the 55 mgN/l. As the concentration of nitrate in a flask reached zero, an additional 20 mg/l NO_3-N were added. In this particular study, GSY 0855 water was "respiked" three times, and the cultures from the other three sumps only once. By the end of day 14, the algae growing in GSY 0855 water had assimilated a total of 105 milligrams NO_3-N, as compared to only 60 milligrams assimilated in the remaining sumps. The cells grown in GSY 0855 tile drainage were practically all one-celled Scenedesmus, whereas a considerable number of Oscillatoria and diatoms appeared in the other water samples.

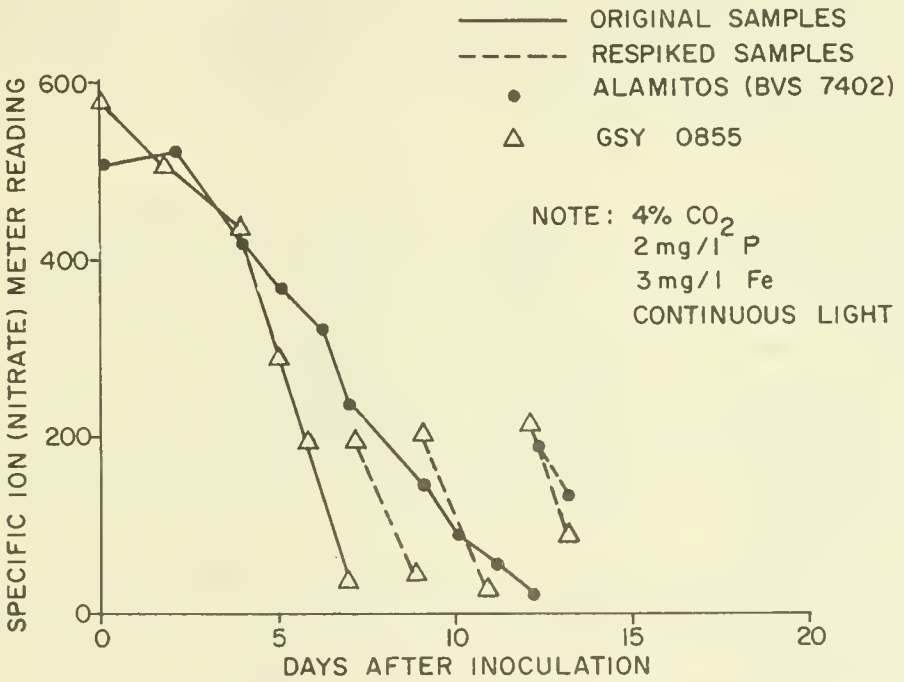


FIGURE 17 - COMPARISON OF NITROGEN ASSIMILATION BY SCENEDESMUS CULTURES USING WATER FROM THREE DIFFERENT TILE DRAINAGE SYSTEMS

The water for the previous study was collected in early September when sump flows were characteristic of summer irrigation practices, especially rice flooding. Another series of samples from the same tile systems was collected in October when field irrigation had been discontinued. The plots of nitrate meter reading versus time for cultures grown in the three tile drainages are shown in Figure 18. Complete nitrate assimilation in all the sumps required about 12 days or about the same length of time required by cultures in DPS 1367, HMH 7016, and BVS 7402 water in the previous study. Results using water from GSY 0855 were similar to those from the other sumps. Algae grown in a composite sample from the four systems to which iron and phosphorus had been added but which contained only about 30 mgN/l, assimilated essentially all of the nitrogen by day 7. The difference in time needed to reach zero nitrate between the composite and the individual sumps undoubtedly was caused by the original difference in nitrogen content of the samples. The individual drainage samples were all brought up to about 55 mgN/l as nitrate.

More studies of this type are needed for confirmation; however, seasonal variation in the algal growth potential of individual tile systems does occur, at least in laboratory cultures. The water used in this investigation appears typical of other systems, and the nutrient additions required for growth of Scenedesmus probably will be required in a combined system.

Miscellaneous Lightbox Studies. Additional lightbox studies were designed to answer specific questions about occurrences in the outdoor growth units. The results of these studies will be presented in later discussions.

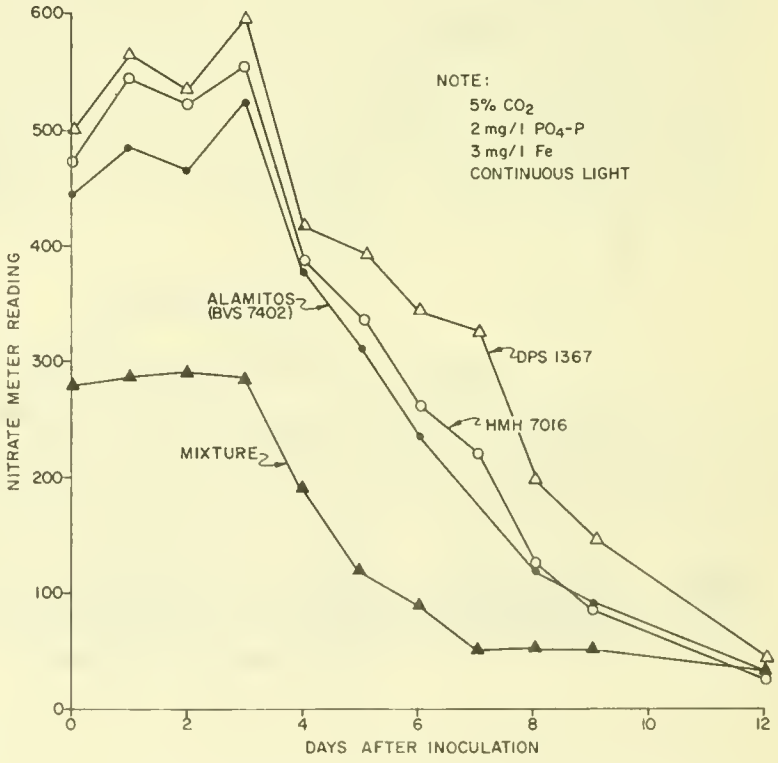


FIGURE 18- COMPARISON OF NITROGEN ASSIMILATION IN VARIOUS TILE DRAINAGE SYSTEMS- OCTOBER SAMPLE

The use of laboratory batch assays can provide valuable information about such items as nutrient requirements, desirable algal species, and comparisons of various waters. However, some discretion is needed in interpreting the results of such studies, especially with respect to nutrient additives. Because environmental conditions in laboratory cultures are near optimum, growth rates are high and the effect of minor elements can often be dramatic. Outdoor cultures are often limited by light or temperature; therefore, the algal cultures do not achieve maximum potential growth and the effect of a minor element is masked. This condition appeared to be especially true with iron, an element necessary for maximum nitrogen assimilation in practically every lightbox study but only seasonally required in outdoor cultures.

Minipond Studies

The miniponds were constructed during the spring of 1968 and were placed in operation that May. Listed in Table 7 are the minipond runs, their duration, and the respective areas of emphasis. During the first three runs, operational problems were being solved, and it was not until fall that adequate physical control of the ponds was achieved. Because of this, results of minipond runs before September 1968 will not be emphasized in this report. This section deals with the effect of nutrient additions and physical and biological parameters on the uptake of nitrate-N.

Nutrient Additions. Five nutrients which, based on the composition of the tile drainage, theoretically limited algal growth were added to the outdoor growth units -- phosphorus, iron, inorganic carbon (CO_2), manganese, and potassium. Of these five nutrients, only the addition of the first three enhanced algal growth and nitrogen assimilation. Manganese and potassium had no demonstrable effect. With all these additions, an attempt was made to maintain a culture of *Scenedesmus quadricauda*. The specific effects of each nutrient are described in the following paragraphs.

Phosphorus. Preliminary laboratory culture studies had indicated that the phosphorous concentration in tile drainage water was limiting to algal growth. In the first three minipond runs, all of four to six weeks duration, the effect of four levels of P addition was evaluated. The results of these studies, shown in Figure 19, demonstrated that phosphorus also was necessary in outdoor cultures of *Scenedesmus*. The optimum phosphorous concentration was not determined. In runs 1 and 2, nitrate removal increased with phosphorous additions up to 2 mg/l P. However, in run 3, only the cultures to which 0.5 mg/l of phosphorus had been added assimilated more nitrogen than did the control which contained no additional phosphorus. During these three runs,

TABLE 7

MINIPOND RUNS CONDUCTED AT
THE IAWTC, 5/15/68 to 12/31/69

Run No.	Dates	Main Areas of Study
1	5/15 to 6/28/68	Phosphorous addition, depth, detention time, fish, soil ponds
2	7/1 to 7/23/68	Phosphorous addition, depth, detention time, fish, soil ponds
3	8/5 to 9/17/68	Mixing, P addition, detention time, depth, fish, soil ponds
4A	9/17 to 12/7/68	Mixing, fish, soil ponds
4B	12/8/68 to 1/25/69	Mixing, fish, soil ponds
5	2/10 to 4/7/69	Mixing, depth, detention time, soil ponds, fish
6	4/16 to 6/27/69	CO_2 addition, Fe addition, depth, soil ponds
7	6/27 to 7/29/69	CO_2 addition, Fe addition, biomass control, detention time, depth, soil ponds
8	8/8 to 9/29/69	CO_2 addition, Fe addition, biomass control, detention time, depth, soil ponds
9A	10/4 to 11/19/69	CO_2 addition, Fe addition, biomass control, detention time, depth, soil ponds
9B	11/19 to 12/31/69	CO_2 addition, Fe addition, biomass control, detention time, depth, soil ponds

influent flows were not regulated with the desired accuracy; therefore, the contradictory results in the third run were assumed to be caused by operational problems.

Beginning in the fall of 1968 and through July 1969, approximately 2 mg/l P (added as H_3PO_4) were added to the storage pond. During the remainder of 1969, all of the miniponds except one received daily P additions. Total nitrogen assimilation for the control pond in one minipond run and a pond at the same depth, detention time, and so forth, are shown in Figure 20. The original inoculum for both of these ponds had been grown in a phosphorus-rich medium (drainage water plus 2 mg/l P), and laboratory analyses of the filtered inoculum indicated that the residue contained about 4 percent phosphorus. Additional analyses of filtered and unfiltered pond samples indicated that a substantial portion of the phosphorus in the residue was composed of colloidal precipitates caused by high in-pond pH values. The data in Figure 20 show that for a two-month period the nitrogen assimilation in the non-P pond was consistently 10 to 20 percent lower than that in the pond receiving supplemental phosphorus. By the middle of December, this difference had increased to

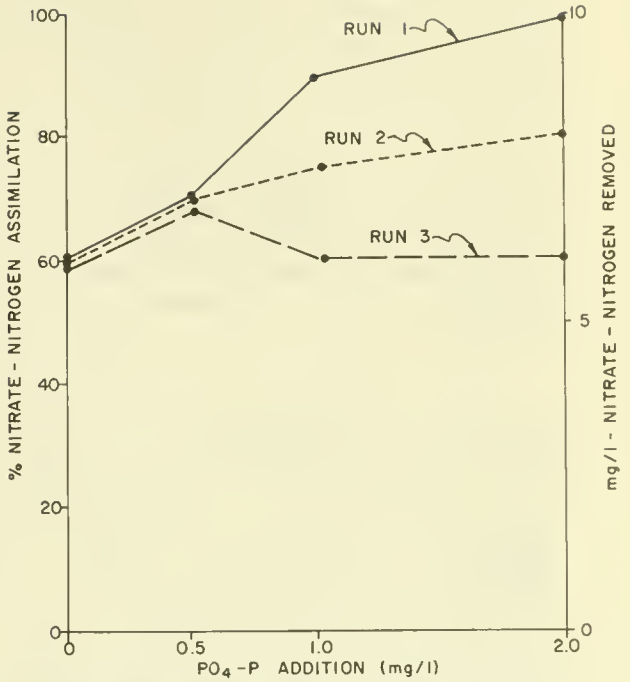


FIGURE 19 - THE EFFECT OF PHOSPHATE ADDITION ON NITRATE ASSIMILATION IN OUTDOOR GROWTH UNITS

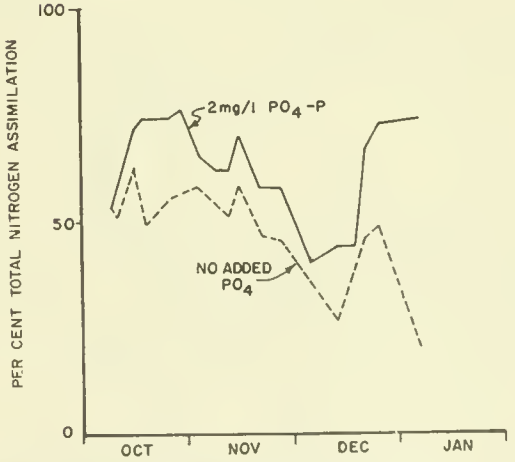


FIGURE 20 - COMPARISON OF NITROGEN ASSIMILATION IN MINIPONDS WITH AND WITHOUT THE ADDITION OF 2 mg/l PO_4-P

30 to 40 percent, perhaps because all excess cellular phosphorus, and all precipitated phosphorus in the pond, had been used. The phosphorus-deficient cultures were pale green and tended to settle more rapidly than those receiving P. Microscopically, the algae seemed to be viable although they lacked the dark green color of typical healthy cells.

Iron. In almost all of the lightbox studies, the addition of 2 to 4 mg/l iron was necessary for maximum nitrogen removal. Results of the first test of this element in the miniponds, performed in the spring of 1969, indicated that substantially more nitrogen could be removed by cultures with 6 mg/l Fe added (using $FeCl_3$ as the source) than by controls with no Fe added (Figure 21). The increase in removal amounted to about 3 mg/l N at the detention times tested. The iron-supplemented cultures were characterized by a dark, luxuriant green color, and microscopic examination of a pond sample showed large numbers of empty cell walls, indicating rapid cell division. As in the lightbox cultures, the use of EDTA seemed to decrease the amount

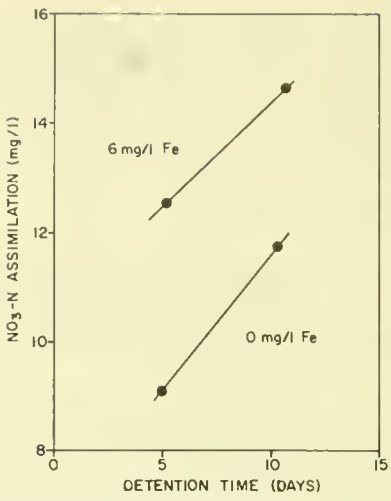


FIGURE 21 - EFFECT OF FE ADDITIONS ON ALGAL NITRATE ASSIMILATION IN OUTDOOR GROWTH UNITS IN LATE SPRING

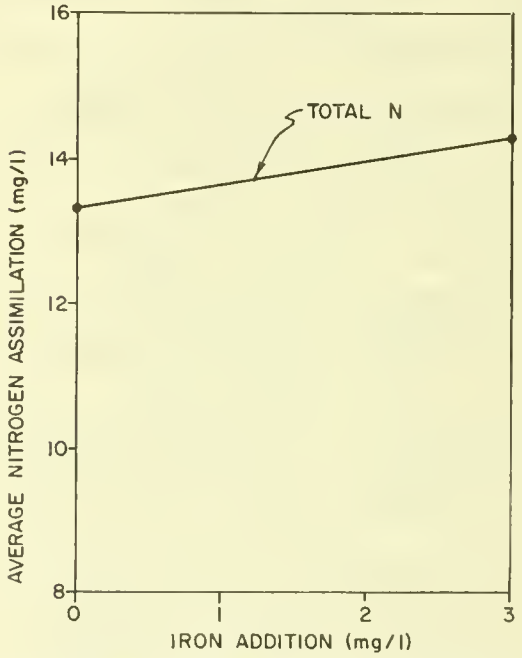


FIGURE 22-EFFECT OF FE ADDITION (3 mg/l) ON ALGAL NITROGEN ASSIMILATION IN OUTDOOR GROWTH UNITS IN LATE SUMMER

of iron required for maximum effect, but its use was discontinued because of the dissolved organic nitrogen contributed by the compound. (Since EDTA contains about 10 percent nitrogen, the daily addition of 25 mg/l EDTA resulted in an increase of about 2 mg/l in effluent dissolved organic nitrogen.) Wide use of this chelating agent in an operating treatment plant probably would not be economically feasible.

From July through December 1969, one minipond was always operated without added iron while the remaining ponds usually received a daily dose of 3 mg/l Fe. The addition of iron had little apparent effect on nitrogen uptake. Illustrated in Figure 22 are the results of a typical minipond run during this period. Although the average total nitrogen assimilation was slightly higher when iron was added, the increase was less than 0.05 mg/l, and was of doubtful significance. Analyses for total (particulate plus dissolved) and dissolved iron in this and other runs provided interesting results. Since the dissolved iron in all samples, including one containing tile drainage only, and another from a pond which received 3 mg/l Fe were essentially the same, the solubility of the element must have been extremely limited. Conversely, total iron concentrations increased in those ponds receiving added iron with the result that more than 30 mg/l Fe was present in many samples, presumably accumulating in the ponds as an iron phosphate precipitate. Because of the low solubility of Fe in tile drainage, the method of addition used in the studies reported herein was extremely inefficient and should receive more consideration before being used in future studies. A beneficial side effect resulting from iron addition was that the $FeCl_3$ acted as a coagulant, thereby reducing the amount of coagulant required in separation studies. This effect is discussed further in the harvesting portion of the report.

Carbon Dioxide. Addition of CO_2 was first evaluated early in the summer of 1969, with rather spectacular results. One hundred percent CO_2 was added to two miniponds by bubbling the gas into the intake side of the mixing pumps during the afternoon mixing session. Results obtained with one of the ponds are illustrated in Figure 23. Almost immediately after CO_2 injection, inorganic nitrogen and orthophosphate concentrations decreased to near zero. For the next few days the culture assimilated all the available inorganic nitrogen and then turned brown and appeared to die. Similar results were noted in the other minipond and in the RGP. In the second minipond, the period of complete assimilation was about twice as long as it was in the previous pond. Injection of CO_2 was discontinued when the culture began to turn brown. Within four weeks after cessation of CO_2 injection, the algae (Scenedesmus) had regained their

FIGURE 23- EFFECT OF CO_2 ADDITION ON EFFLUENT QUALITY IN OUTDOOR GROWTH UNITS IN LATE SPRING

original color and metabolic activity. Monitoring the in-pond pH indicated that the pH level never fell below seven, although it may have been much lower at the point of injection.

The minipond runs following the initial CO_2 studies all included various levels of CO_2 addition -- 100 percent, 50 percent, 5 percent, atmospheric air, and natural aeration. In general, the results of these runs indicated that the addition of CO_2 increased nitrogen uptake and that a 5 percent CO_2 concentration was preferable to 100 percent. Table 8 contains average nitrogen assimilation data from

TABLE 8

AVERAGE PERCENT TOTAL NITROGEN ASSIMILATED FROM 20 mg/l INFLUENT (MINIPOND RUN 8)

Detention Time : Days	Type Addition			
	5% CO_2	100% CO_2	Air	Natural Aeration
3	43	52	46	
5	64	62	60	49
8	78	74	70	

minipond run 8, which lasted from August 6 through September 29, 1969. These data show that at a three-day detention time nitrogen assimilation was low regardless of the availability of CO_2, indicating that detention time, not carbon, was the limiting factor. At five days detention time, 5 percent CO_2, 100 percent CO_2, and atmospheric air were all somewhat more effective than was natural aeration. At eight days detention time, about 8 percent more nitrogen was removed in cultures receiving 5 percent CO_2 than in those receiving atmospheric air only.

Another example, based on minipond run 9A (October 4 through November 19, 1969) is illustrated in Figure 24. In this run, when the detention period was held at 11.4 days, an average of about 80 percent of the influent nitrogen was assimilated by cultures injected with 100 percent and 5 percent CO_2; whereas only about 55 percent was removed by cultures not receiving CO_2 enriched air. When the detention period was reduced to eight days, nitrogen uptake seems to have been enhanced only when 5 percent CO_2 was added. With further reduction of detention time to five days, assimilation was not affected by the presence or absence of CO_2 enrichment. An interesting aspect of the experiment in which 100 percent CO_2 was added to one of the

ponds was the sudden decline in nitrogen uptake after November 9 (Figure 24). At this time the rate of algal growth in the pond seems to have declined, although the culture appearance remained green and healthy. This pond continued to receive 100 percent CO_2 until December but never regained its former assimilation rate.

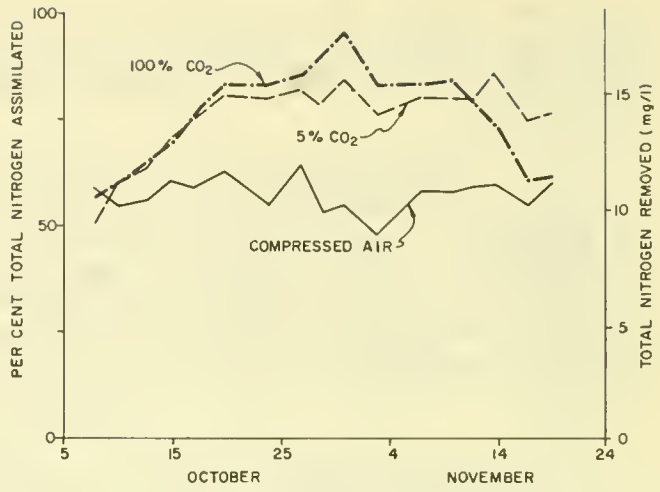


FIGURE 24- EFFECT OF DIFFERENT LEVELS OF CO_2 ADDITION ON NITROGEN ASSIMILATION IN PONDS AT 11.4 DAYS DETENTION TIME

The apparently active toxic effect of 100 percent CO_2 demonstrated in the early summer of 1969 was never again noted in subsequent runs. Possible reasons for this effect may have been toxicity of the gas itself or toxicity of material brought into solution by the lower pH values. When the apparent toxic effect was first noted in the outdoor cultures, samples of pond water and CO_2 were taken into the laboratory for lightbox culture studies. No toxicity was evident when filtered pond water was assayed for this factor with both 4 percent and 100 percent CO_2. Another possible explanation for the die-offs was based on the hypothesis that the algae died because of their rapid growth rate resulted in nutrient starvation. This hypothesis was tested by allowing laboratory cultures to remove all of the available nitrogen from the medium and leaving them in this nutrient-deficient condition for up to 10 days (with continuous lighting). At the end of this starvation period, the cultures were given nitrogen, iron, and phosphorus. The algae readily incorporated the additional nitrogen and assumed a healthy appearance. The true cause of the apparent CO_2 toxicity is still unresolved and, since the effect was never repeated after the first few occurrences, the question may never be answered. Minipond runs in late 1969 did indicate that the addition of CO_2 was required to achieve maximum nitrogen assimilation in the outdoor cultures, but that the quantity of CO_2 which is optimum will depend on season and drainage water quality.

Mixing. The exact role that mixing plays in outdoor cultures may be a combination of various effects including: (1) moving the algae into the light; (2) reducing anaerobic

sludge banks caused by settled algae; (3) removing the algae from the system by keeping them in suspension and thus available for discharge in the effluent; (4) replenishing of carbon dioxide by increasing the surface area exposed to the atmosphere; and (5) by stirring the bottom deposits, making essential nutrients more available. In the first two minipond runs, mixing was limited to sweeping the ponds twice daily. In these studies, actively growing cultures of Scenedesmus often completely settled from suspension during the afternoon, presumably as a result of entrapment in precipitated calcium and magnesium salts at pH values above 10. In minipond run 3, power and equipment became available for mixing six miniponds. The results, shown in Figure 25, indicated that the amount of nitrogen assimilated could be significantly increased by continuously mixing the cultures.

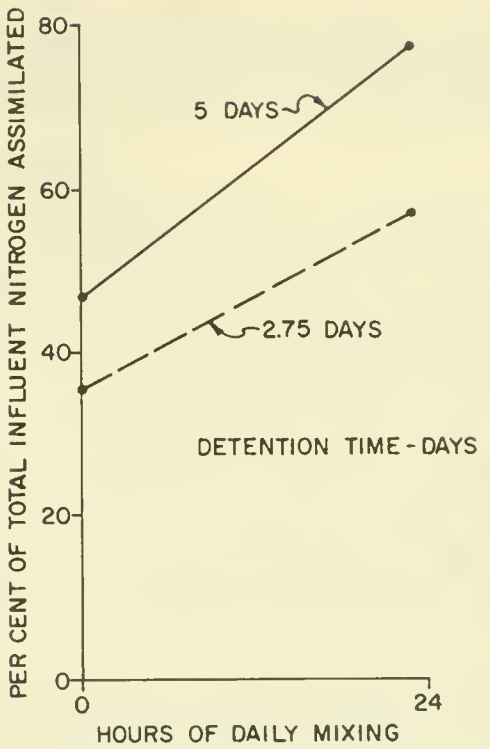


FIGURE 25- EFFECT OF CONTINUOUS MIXING ON NITROGEN ASSIMILATION, MP RUN 3

By the fall of 1968, all but two of the miniponds had mixing systems, and in run 4 various durations of mixing were investigated. The average percentages (each number is an average of three ponds) of total nitrogen assimilated for approximately 60 days operation are plotted in Figure 26. The two- and four-hour mixing periods took place in the afternoon, and the 12-hour period occurred during daylight hours. The results showed that total nitrogen assimilation was almost doubled by the optimum amount of mixing and that 4 and 24 hours had approximately equal effects. The unexplained decrease in nitrogen removal in cultures mixed 12 hours daily noted in the individual weekly averages was unexplained but statistically significant. During the run, total nitrogen removals were low in all the ponds; therefore, the study was repeated on a smaller scale in May and June, 1969. The main emphasis at that time was placed on determining what portion of the daylight hours was optimum for

mixing the cultures. The results of the four mixing periods, four hours in the morning, four hours in the afternoon, 15 minutes of each daylight hour and for 14 daylight hours, are shown in Figure 27. After the initial period of adjustment,

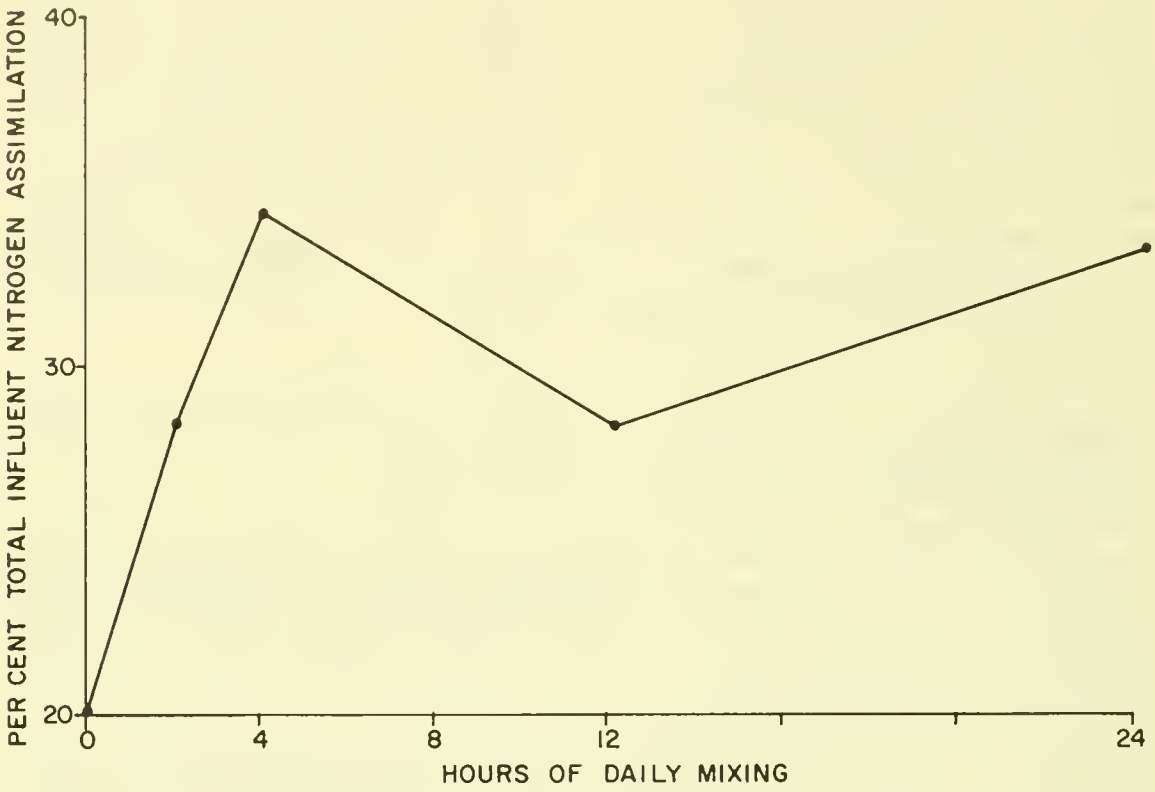


FIGURE 26-EFFECT OF MIXING DURATION ON AVERAGE TOTAL NITROGEN ASSIMILATION IN MINIPOND RUN 4A

up to about May 20, four-hour mixing in the afternoon consistently increased the amount of nitrogen incorporated by the algal cultures. Full daylight mixing, either continuously or for 15 minutes of each hour, was detrimental to nitrogen assimilation. Again, in July 1969 cultures continuously mixed during daylight hours assimilated less nitrogen (65 percent) than did cultures with afternoon mixing only (85 percent), both at eight days detention time.

Based on the information from the runs cited above, the standard mixing regimen imposed was four daylight hours. For convenience in sampling, this period was divided into two intervals -- 0800 to 0830 hours and 1200 to 1530 hours. One effect of this regimen imposed on the miniponds was to periodically change the type of flow through the system. Tracer

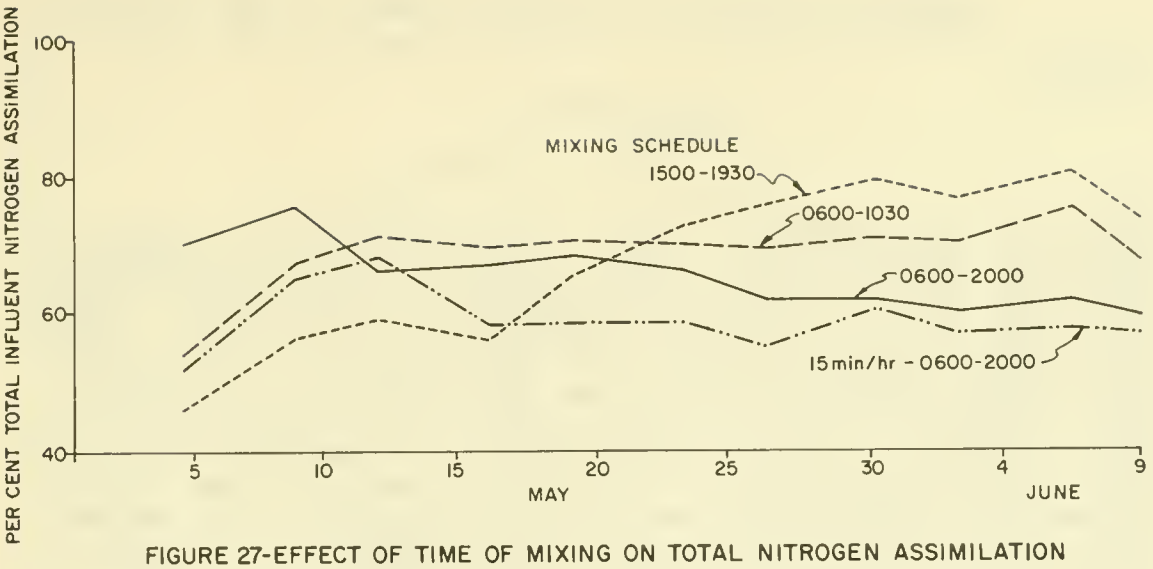


FIGURE 27-EFFECT OF TIME OF MIXING ON TOTAL NITROGEN ASSIMILATION

studies showed that the pond influent went through the pond as a discrete entity (plug flow) when the mixing pumps were off. During mixing periods, the ponds acted as almost completely mixed vessels.

The average in-pond mixing velocities were determined by measuring the velocity at various points in some selected ponds. These results showed that the average velocities varied from about 0.25 feet per second (fps) in the 16-inch pond to about 0.50 fps in the 8-inch ponds. These velocities did not prevent the algae from settling, especially in some stagnant areas of the pond. The two ponds that were modified to eliminate these stagnant spots (see Figure 5) had higher velocities than comparable unmodified ponds, and they were relatively free of algal sludge banks. In spite of the increased mixing efficiency, no significant difference occurred in rates of nitrogen assimilation between algal cultures grown in the two types of pond.

After the addition of CO_2 became routine, one minipond was left without CO_2 and without mixing. The data from this unit, when compared to a similar pond that was mixed, showed comparable effluent dissolved nitrogen levels, a finding that indicated mixing was not always essential to algal growth. Preliminary evaluation of the effect of CO_2 addition to a nonmixed pond has indicated that CO_2 alone may be adequate for achieving the desired rates of nitrogen uptake. The mixing requirements of an algal stripping system will receive more emphasis in the 1970 operational studies.

Detention Time. Detention times as used in this study are theoretical hydraulic terms computed by the formula:

$$\theta = \frac{V}{F}$$

where θ is the detention time, F the flow, and V is the volume of the container. Three separate dye tracer studies indicated that the true and the theoretical hydraulic detention times were in reasonable agreement. For example, the calculated theoretical detention time for one of the ponds was 5 days and the measured true detention time about 5.2 days. Because of pond design, mixing, and natural algal settling rates, cell detention time was significantly longer than the hydraulic detention time. In one instance, when the two were compared by use of tracer studies, the values were about 30 days for the cells, as compared to 5 days for a hypothetical water molecule (periods of mixing, 0800 to 0830 hours and 1200 to 1530 hours).

The effect of detention time can be best evaluated from results obtained after July 1969 because standard mixing, nutrient additions, and detention times were used during this period. Detention times of 3, 5, 8, 11.5, and 16 days were studied, although only three of these times were compared in any particular run. An example of a detention time study is illustrated in Figure 28. The ponds were operated on a batch basis until August 15, 1969, when the desired detention times were set. After reaching steady-state, the pond with an eight-day detention time consistently assimilated 80 percent or more of the influent nitrogen. At five days, steady-state was reached at a level less than 75 percent, and at three days the level was closer to 50 percent total nitrogen uptake. The sudden decrease in removal that occurred about September 12 may have been caused by a sudden temperature drop during this period. (See Figure 48.)

The overall effect of hydraulic detention time is best illustrated in Figure 29, in which the data from four minipond runs from July through December 1969 are summarized. The data are averages of total nitrogen assimilations for 12-inch ponds in which CO_2 was the only other variable tested, i.e., depth, mixing, etc., were identical. As will be shown in later sections, detention times can also be affected by physical changes in the system. The influent total dissolved nitrogen concentration was about 20 mg/l during this period. The results demonstrate clearly that detention times must be varied seasonally to achieve the desired algal growth. The average maximum removals of dissolved influent nitrogen for each run (which included start-up) were consistently near

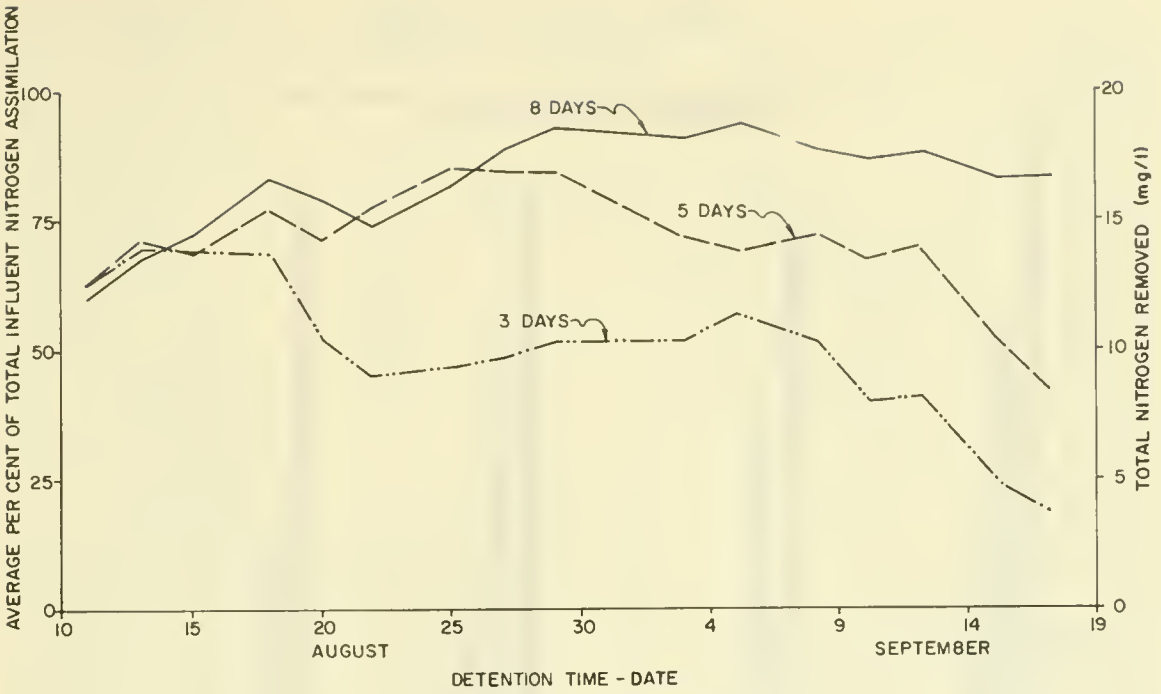


FIGURE 28 - EFFECT OF DETENTION TIME ON NITROGEN ASSIMILATION, 5% CO_2 ADDED

80 percent, while in the actual runs the steady-state levels were in the 85- to 95-percent range. In these 12-inch depth ponds, eight days was the shortest detention time which would allow for the assimilation of an average of 80 percent of the influent nitrogen. Although not shown in this figure because of differing mixing schedules and the absence of carbon dioxide, data for May and June also indicated that detention times of less than eight days were too short for maximum nitrogen assimilation.

Required detention time for a given rate of nitrogen assimilation is intimately related to water temperature and must be adjusted to ensure that the rate of nitrogen assimilation is not significantly different from nitrogen input. Smooth curves of the maximum and minimum daily minipond temperatures are shown in Figure 30. Actual maximum and minimum water temperatures recorded were 98°F (36.6°C) and 34°F (1.1°C) respectively. The average maximum temperature for each run was calculated from these curves. (Because the curves had generally similar shapes, minimum temperatures could also have been used.) In Figure 31, shows the average maximum temperatures versus the detention time required to remove about 16 mg/l of the total influent nitrogen.

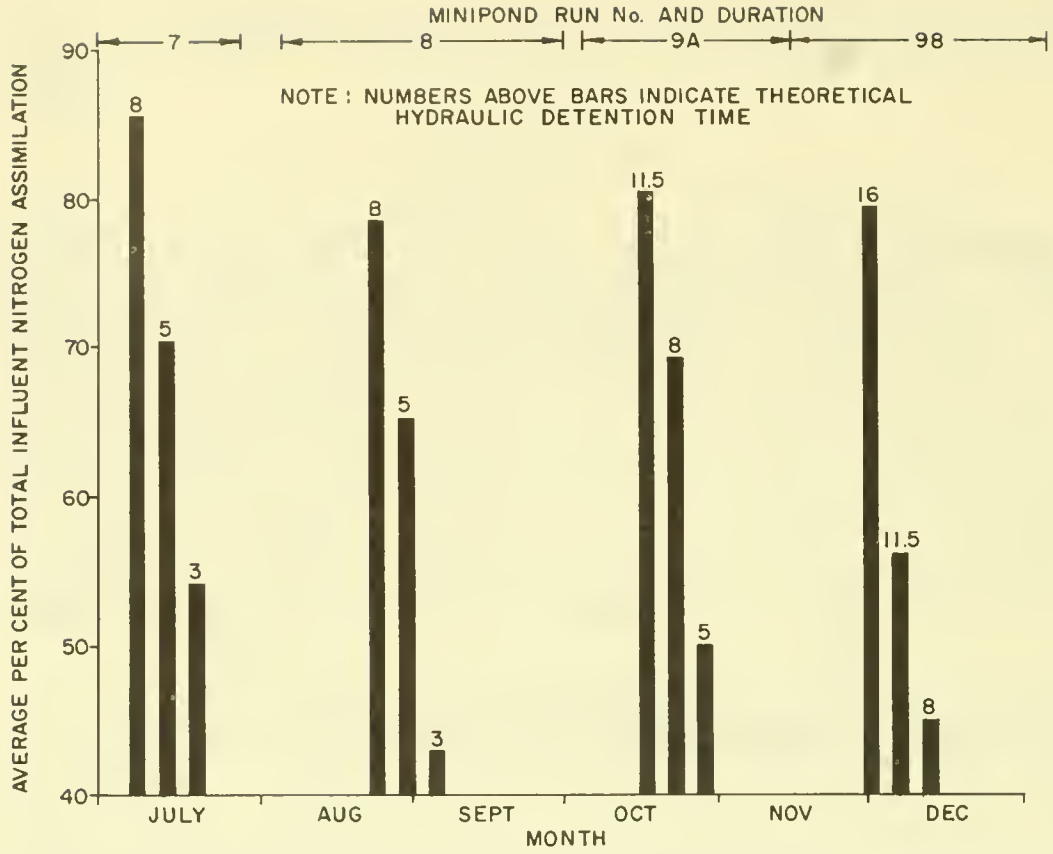


FIGURE 29-EFFECT OF DETENTION TIME ON NITROGEN REMOVAL-SUMMARY OF DATA FROM 12 INCH PONDS FROM JULY-DECEMBER 1969

From these data it appears that detention time was indirectly proportional to maximum average pond temperature between 15 and 25°C, the range of temperatures during the selected study period. With standardization of pond operation and shorter run times during the operational studies, such a curve will be extended to cover the entire range of temperatures found on the west side of the San Joaquin Valley.

Culture Depth. Water depths of 8, 12, and 16 inches were studied as part of the investigation. Depth primarily affects amount and quality of available light energy received by algal cultures and indirectly affects mixing. As shown previously, the average water velocity in the 16-inch deep pond was about 0.25 fps as compared to 0.5 fps in an 8-inch pond. Experiments conducted during the period of May through December 1969 were taken as the source of data for studying this variable, because of the uniform operating conditions during that time. Because of the limited pond availability, only the 12-inch depths were replicated in any particular run. The data for the 8- and 16-inch depths were from single ponds. In all minipond runs, nitrogen

assimilation in the 8- and 16-inch culture depths differed distinctly. The curves in Figure 32 show the typical effect of depth. All these ponds were operated at eight days detention time and received 5 percent CO_2. The overall average assimilations of total influent nitrogen were 80, 69, and 55 percent for 8, 12, and 16 inches, respectively.

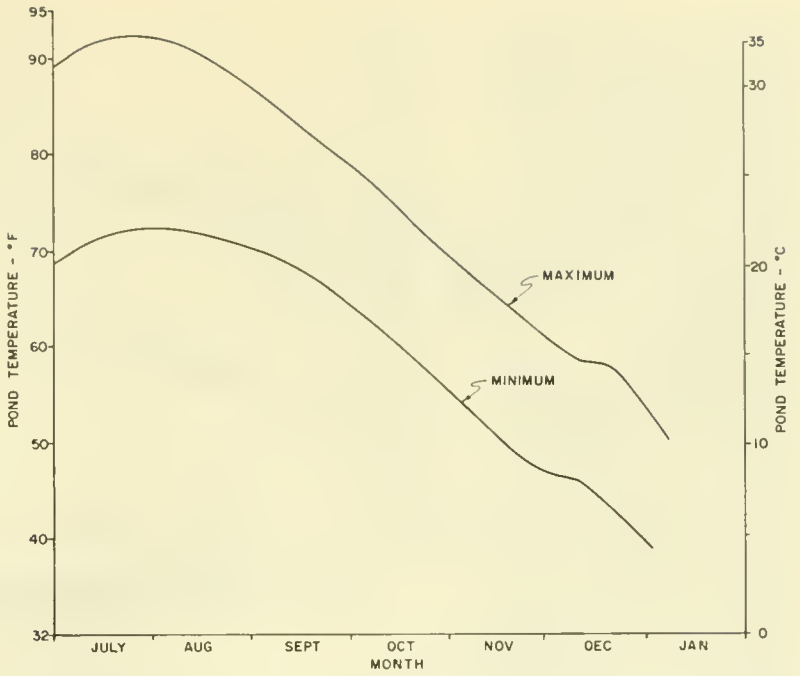


FIGURE 30 - MAXIMUM AND MINIMUM MINIPOND TEMPERATURES - JULY TO DECEMBER 1969

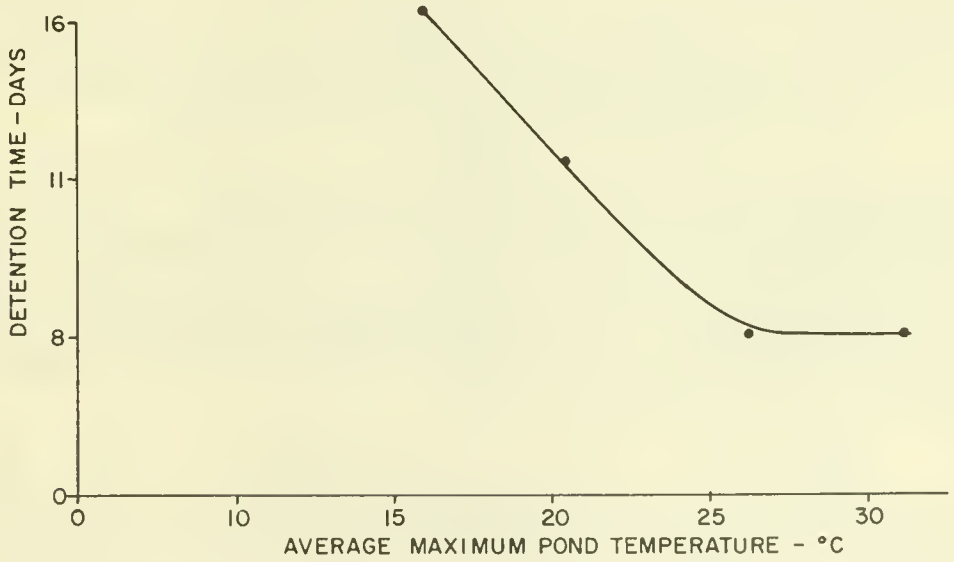


FIGURE 31 - PLOT OF DETENTION TIME REQUIRED TO ASSIMILATE APPROXIMATELY 80% OF THE TOTAL NITROGEN vs AVERAGE RUN TEMPERATURE

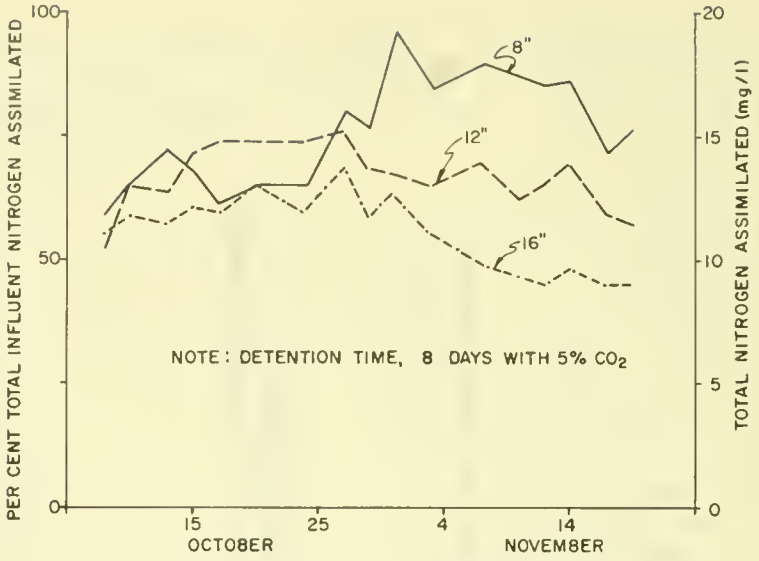


FIGURE 32 - EFFECT OF THREE DIFFERENT CULTURE DEPTHS ON TOTAL NITROGEN ASSIMILATION

All of the average total assimilations for each depth were plotted for the five minipond runs, Figure 33, and the slopes (change in percent removal per change in depth, $\Delta\%R/\Delta D$) of the straight lines from the percentage at 8 inches to that at 16 inches were calculated, and the values listed in Table 9 were obtained. The data show that the slope of the line increased as the days became shorter, and that the difference in nitrogen assimilation between algae cultures grown at 8 and 16 inches of depth was more pronounced when available light energy per day increased.

In Figure 34 the slopes from Table 9 have been plotted as a function of the average daily solar radiation during each of the runs. A straight line fitted by eye indicates that the effect of depth within the limits tested was directly correlated to average daily solar radiation.

As illustrated in Figure 35, one effect of decreased depth is to lessen the detention time required for algal assimilation of the available nitrogen. In the four runs shown, the average amounts of nitrogen assimilated were practically identical; however, in each run the necessary detention times were three to four days shorter in the 8-inch culture depths. The savings in detention time are about balanced by the additional land area required for treatment at the shallower depth.

Soil Ponds. The two ponds which had layers of soil on the bottom received a minimum of operational attention, consisting mainly of regulation of the influent flow and addition of phosphorus, and they were never drained or refilled. In spite of, or perhaps because of, the lack of attention, both ponds maintained cultures of organisms which removed surprisingly large amounts of nitrate-nitrogen. Both ponds had a mixed biota consisting of algae, zooplankton,

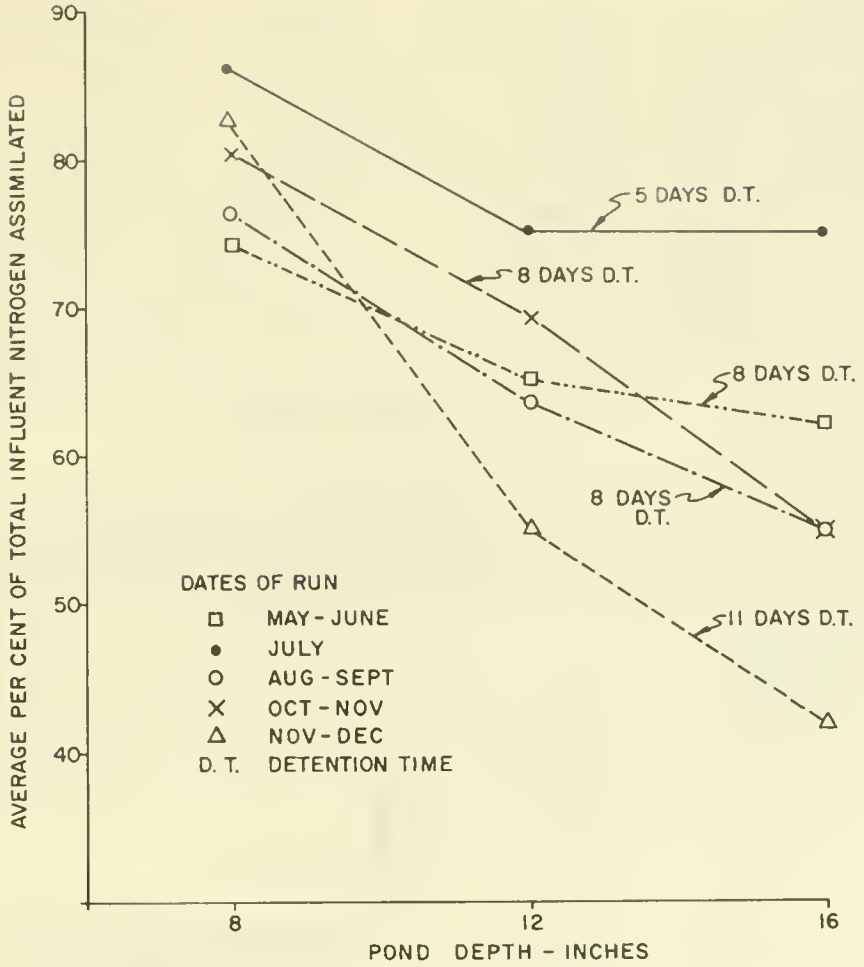


FIGURE 33- AVERAGE TOTAL NITROGEN ASSIMILATION FOR VARIOUS CULTURE DEPTHS, MINIPOND RUNS 6-9B

TABLE 9

SLOPE OF VARIOUS DEPTH VERSUS PERCENTAGE REMOVAL CURVES

Run	Approximate Dates of Run	Slope (Δ %R/ΔD)*
6	May-June	1.5
7	July	1.5
8	August-September	2.5
9A	October-November	3.1
9B	November-December	5

*Percent change in removal per change in depth from 8 to 16 inches.

higher aquatic plants, bacteria, and flying insect larvae (mainly dipteran). Dissolved oxygen determinations made at different times of the year indicated that the soil ponds were always aerobic, at least above the soil-water interface. The amount of algae was usually low (less than 100 mg/l), and the algae were often of the flagellated variety, e.g., Euglena and Carteria. An indication that the nitrate removal

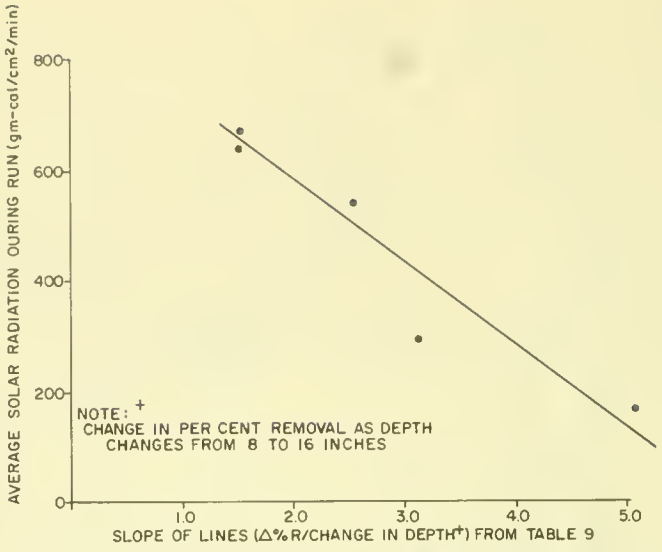


FIGURE 34-SLOPE OF DEPTH vs PER CENT REMOVAL CURVES COMPARED WITH AVERAGE SOLAR RADIATION DURING CORRESPONDING RUN

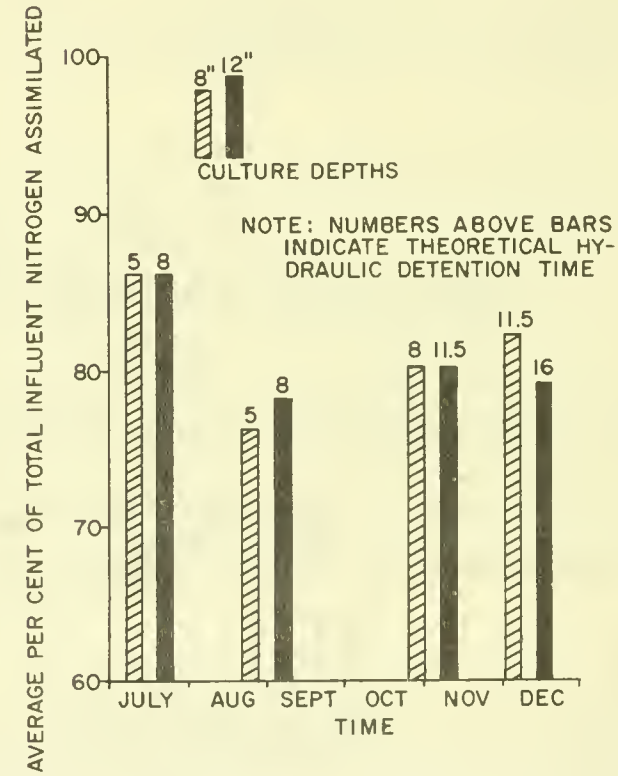


FIGURE 35-COMPARISON OF AVERAGE TOTAL NITROGEN ASSIMILATION AT TWO CULTURE DEPTHS, 8 AND 12 INCHES, AND VARIOUS DETENTION TIMES

mechanism was not entirely algal was provided in a study in which a sample of the soil, water, and organisms was enclosed in a darkened bottle and floated in the pond. At the end of one week, the original 21 mg/l of total dissolved nitrogen had been reduced to 14.5 mg/l (NO_3-N, 12 mg/l; NO_2-N, 2 mg/l; and NH_3+Organic-N, 0.5 mg/l). Because light was not available during the incubation period, the nitrogen reduction was assumed to be the result of anaerobic bacterial decomposition. The dissolved oxygen content of water in the bottle decreased from about an initial 12 mg/l to a non-detectable amount at the end of the test period. The organic carbon source was presumably the product of algal and bacterial decomposition, as well as extracellular organic products of algal metabolism.

A comparison of nitrogen removal data from one of the soil ponds with a minipond of comparable depth and detention is shown in Figure 36. The remaining soil pond followed essentially the same pattern as the one shown and was omitted for graphical clarity. Also included in the figure are the removal data for the best 12-inch depth non-soil pond in each minipond run. These data support the following conclusions: soil pond cultures consistently removed more nitrogen than did a mixed minipond at a similar detention time, and the removals in the soil ponds were often higher than in the best routinely operated minipond.

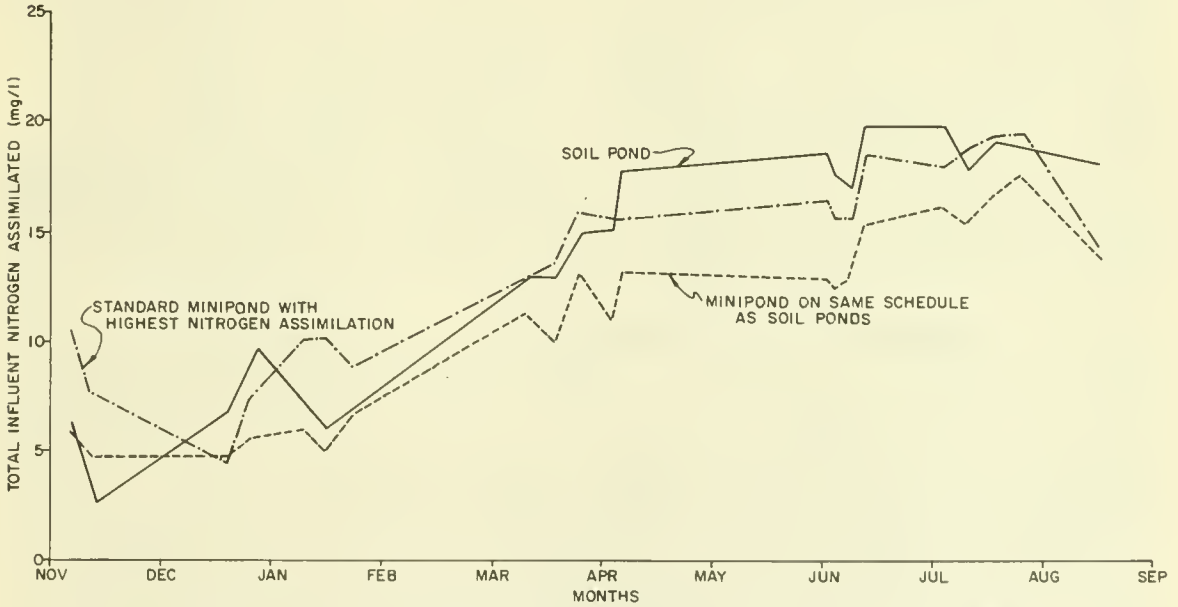


FIGURE 36-COMPARISON OF TOTAL NITROGEN ASSIMILATION IN ONE SOIL POND WITH A COMPARABLY OPERATED MINIPOND AND STANDARD MINIPOND WITH HIGHEST NITROGEN ASSIMILATION

The operational studies in 1970 were designed to define the mechanisms involved in reduction of nitrogen in these ponds, which we have called "symbiotic ponds". The nitrogen removal mechanisms for these units appear to resemble that in grass plots described by Williford and Cardon (1971) in which water flowing through fields of water grass showed marked nitrogen reduction. In the grass plots, relatively little planktonic algal growth took place; dissolved oxygen concentrations were always above zero; and no organic carbon was added. Net influent nitrate concentrations ranging from 30 to 300 mg/l (as NO_3) were reduced by amounts ranging from 50 to almost 100 percent. The authors tentatively concluded that the reduction was the result of anaerobic denitrification in local strata near or at the soil-water interface.

Biomass Control. The term biomass control is used to describe the process of removing settleable algae and other settleable materials which tend to accumulate in the ponds during long periods of operation. Mixing schedule, algal settling, and pond design each contributed to the problem of accumulation of algal cells in the small outdoor ponds. The possibility that the older cells might be detrimental to growth of the younger cells was considered in this study. The detrimental effect could result from light exclusion by older, nonreproducing cells, by the production of some autoinhibitory substance, or by competition for scarce nutrients. Although not a part of the algal biomass, particles of suspended inorganic precipitates also tended to accumulate in the ponds and substantially reduced light penetration into the cultures. To determine whether or not such effects were present and their influence, a settling tank (described previously) was attached to the mixing pump of one of the two redesigned miniponds, while the other pond was operated as a control. As shown by the curves in Figure 37, the settling tank reduced the volatile solids within the pond by about 50 to 60 mg/l during minipond run 9A.

The total nitrogen removal for the two ponds is shown in Figure 38, both at eight days detention time and with 5 percent CO_2 addition. The average total removal for the pond with biomass control was about 80 percent; for the control pond, 61 percent. During this run the best of the remaining 12-inch ponds also removed about 80 percent but at a detention time of 11.5 days. In another run involving five days detention time, the pond with the settling tank removed an average of 71 percent compared to 59 percent removed by the control. These data indicate that accumulation must be prevented in outdoor cultures, and that the construction of larger units may have to include some mechanism for controlling the accumulation of inactive algae in the pond,

such as in-pond settling areas with sludge removal, or other suitable methods. By providing control of excess biomass and inert materials the required detention times for maximum nitrogen assimilation may be lowered significantly. The data from this study of biomass control indicated that, during the period from July through November, the rate of nitrogen assimilation in the biomass-controlled ponds were comparable to other 12-inch ponds which were being operated with about three days of additional detention time. Thus with biomass control, five days may be the minimum summer detention time to achieve 80 percent or more assimilation.

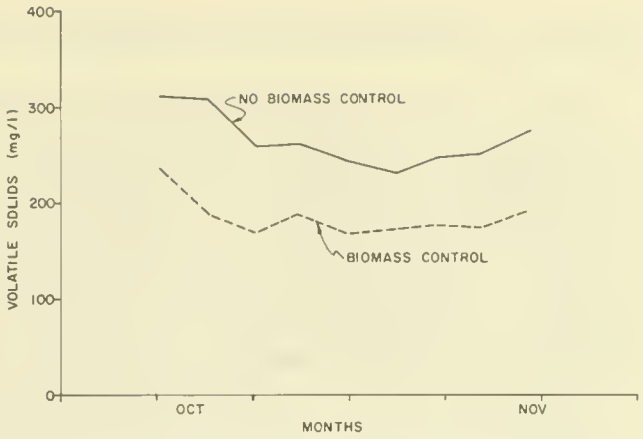


FIGURE 37-COMPARISON OF VOLATILE SOLIDS IN MINIPONDS WITH AND WITHOUT BIOMASS CONTROL

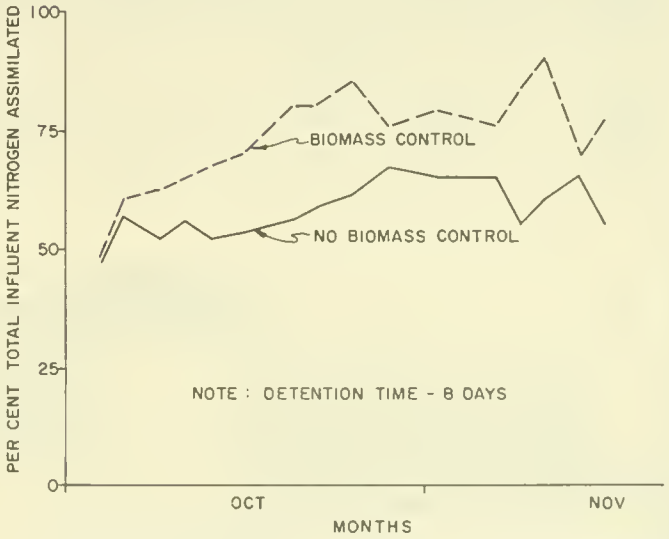


FIGURE 38-PER CENT TOTAL INFLUENT NITROGEN ASSIMILATED IN MINIPONDS, WITH AND WITHOUT BIOMASS CONTROL.

Addition of Fish. Some preliminary studies indicated that fish may possibly enhance the growth of algae in tile drainage waters and aid algal assimilation of nitrogen. Their beneficial effect was thought to be due in part to their stirring action and in part to their production of some waste product essential to algal metabolism. For these reasons, and because fish might also add a true recreational value to drain waters, a quantity of Sacramento blackfish weighing from 0.5 to 1.5 pounds each were obtained in May 1968 and placed in some ponds during the first four minipond runs. Some ponds received 2.5 pounds of fish and some received 5 pounds of

fish. This particular species was selected for its reported active consumption of planktonic algae.

The fish survived in the miniponds, although some mortality was noted. Unfortunately, no record of growth was maintained; thus we do not know if the fish actually gained weight in the ponds. The fish did have a beneficial effect on nitrogen uptake. In Figure 39, which summarizes their impact in the first four runs, the best minipond without fish is compared to a pond with comparable operating criteria containing fish. In all four runs, the algae in the ponds with fish assimilated more nitrogen than did the control ponds. The increased nitrogen assimilation attributed to the presence of fish ranged from 13 to 43 percent with an average of 28 percent. After minipond run 4A, all fish were removed from the miniponds, chiefly because iron additions had proved to be more effective at increasing nitrogen removal but also because the screens covering the influent to the mixing pumps (to keep the fish out) were reducing the mixing efficiency. Large algal ponds can apparently support fish growth and fish apparently do enhance algal growth and nitrogen assimilation.

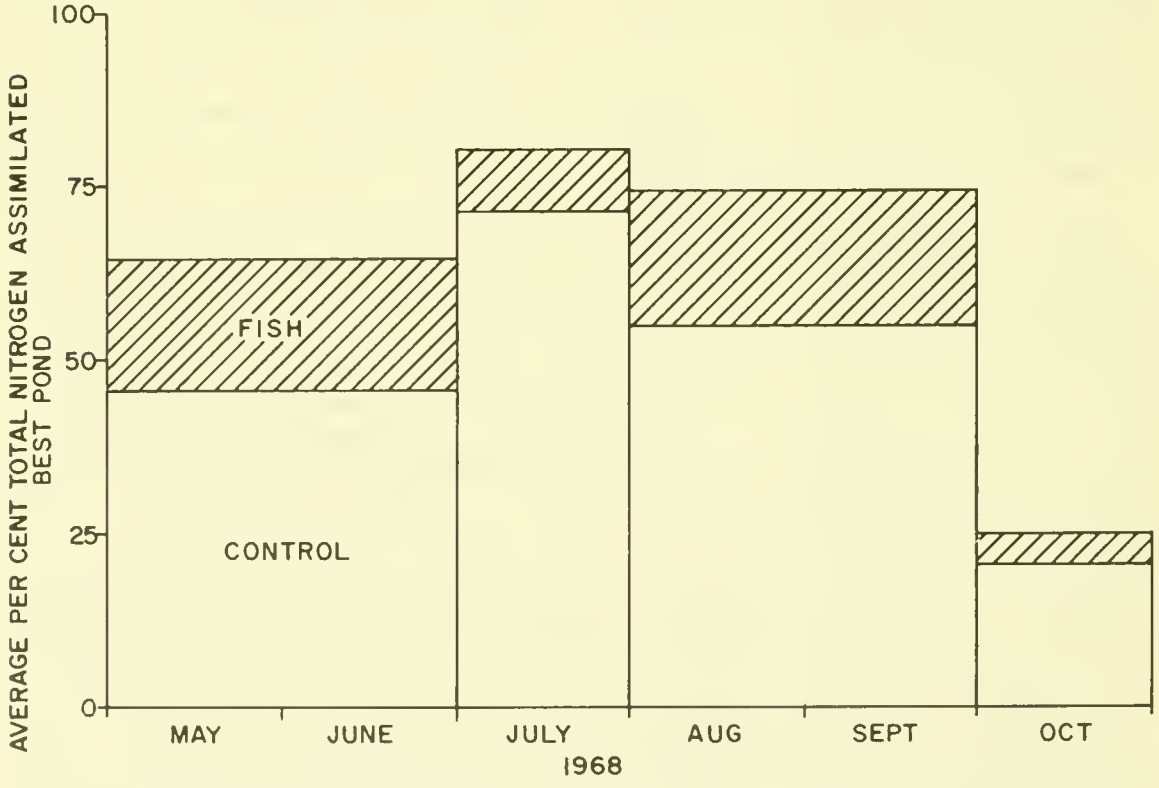


FIGURE 39-PROPORTIONATE INCREASED NITROGEN ASSIMILATION ATTRIBUTED TO FISH, BY SEASON

Rapid Growth Pond

This pond was of limited value as a research tool because of the lack of a comparable unit to use as control; consequently, it was used principally as a demonstration unit and a source of algae for separation studies. During the first few months of operation we were unable to maintain Scenedesmus cultures and the pond was reseeded twice, once in the summer of 1968 and again in January 1969. The pond normally was operated with a 12-inch water depth and at varying detention times, on the basis of minipond data. During these first few months, the pond occasionally was operated at less than 20 mg/l influent nitrogen and with different mixing cycles. After January 1969, the pond was operated for almost five months without being drained and with little variation in operating procedures.

Plots of effluent nitrogen, influent nitrogen, and detention time for the RGP during the calendar year 1969, are presented in Figure 40. The period up to June 12 was characterized by a relatively stable effluent that averaged about 5 mg/l total N (75 percent assimilation). On June 12, 100 percent CO_2 was added to the pond, whereupon the effluent inorganic nitrogen decreased to near zero. Ten days later the pond turned brown and nitrogen removal ceased. On July 7

FIGURE 40- OPERATION OF RAPID GROWTH POND DURING 1969

the pond was drained and cleaned, and on July 14 the pond was refilled and inoculated with the contents of two of the miniponds. The 100 percent CO_2 was again added but with slightly different results than before. Scenedesmus began to grow actively but were soon replaced by diatoms and blue-green algae, which floated to the surface one afternoon and were removed by surface overflow. The Scenedesmus then returned in late July and have since remained as the dominant algae. The possible explanations for this change in species composition could be the apparent CO_2 toxicity discussed earlier in the section on addition of CO_2 to the miniponds.

After July 1969 we were hesitant to try CO_2 again because the Scenedesmus culture in the pond was the principal source of material for the separation studies; and, inasmuch as the addition of 100 percent CO_2 to the pond might have proved lethal or at least inhibitory to the population of Scenedesmus, no CO_2 was added to the pond thereafter. Also, after July the pond was often operated to conform to the needs of the individual separation studies. For example, occasionally the pond was mixed all day to provide a uniform algae supply even though full-day mixing had been found to reduce nitrogen assimilation. Because of this, the nitrogen uptake figures are minimal approximations of the unit's potential. The 1970 operational studies will provide a more realistic evaluation of the potential nitrogen removal capabilities in this pond.

Biological and Chemical Observations

Biomass Production. As used in the present study, the term biomass refers to the results of volatile suspended solids determinations. In many algal growth studies, cell counts, packed cell volume, or light transmittance (or absorbance) are used to estimate changes in biomass. The primary interest at Firebaugh was in biomass because of its possible effect on the growth system and on the market value of the algae produced; therefore, estimates of biomass production were needed. Volatile suspended and total suspended solids of algal cultures can often be used interchangeably since the ash portion of algae is usually only 10 to 15 percent of the total weight. At Firebaugh the volatile portion was usually only 50 to 70 percent of the total suspended material. The nonvolatile solid material consisted of insoluble complexes of magnesium, calcium, phosphorus, potassium, and iron resulting from the high pH levels attained in the growth units, as well as of some windblown soil particles. Although CO_2 addition to the growth units did lower the pH,

apparently no significant change occurred in the percentage of volatile material in the effluent solids (Figure 41).

The mixing system finally selected for general use in the outdoor growth units caused obvious diurnal fluctuations in the effluent volatile solids. A typical diurnal cycle is illustrated in Figure 42. In this particular instance, the 0830 volatile solids (normal sampling time) of 374 mg/l indicated an unrealistically high value for the amount of biomass produced by the system. Graphical integration of this curve indicated that the average volatile solids concentration for the 24-hour period was approximately 120 mg/l. Because of the diurnal fluctuation caused by algal settling, the total pond biomass increased indicating that it was accumulating at a rate faster than that at which it was being removed. Volatile solids concentrations of more than 500 mg/l often were noted in the growth units and the solids seldom reached steady-state conditions.

Actual biomass production during a 19-day period of August-September 1969 was calculated for a minipond in

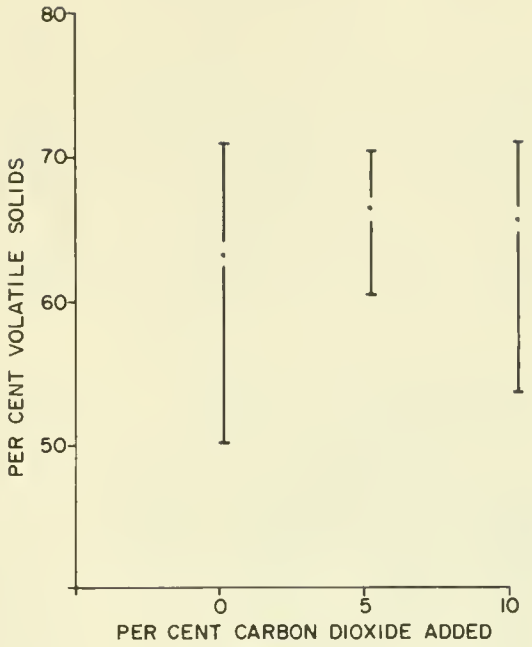


FIGURE 41- AVERAGE AND RANGE- PER CENT VOLATILE SOLIDS OF TOTAL SUSPENDED SOLIDS AT DIFFERENT LEVELS OF CO_2 ADDITION

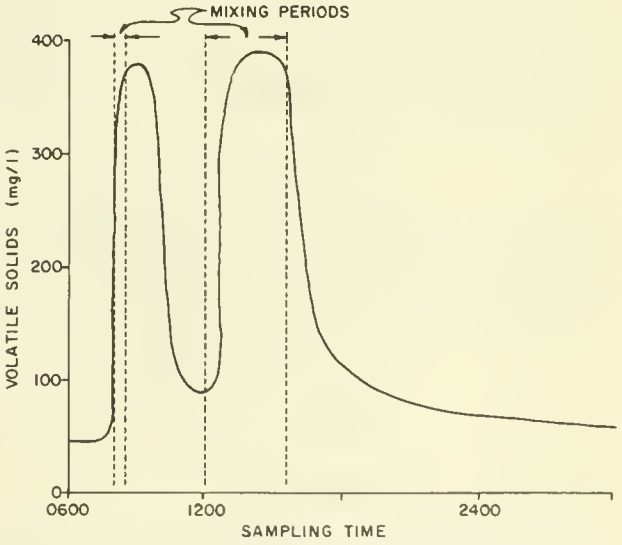


FIGURE 42- DIURNAL FLUCTUATIONS IN EFFLUENT VOLATILE SOLIDS

run 8. Effluent (0830 samples) volatile solids and total nitrogen are shown in Figure 43. An overnight study of the pond during this period indicated that the average volatile solids in the effluent were about one-half of the 0830 values. Both curves in Figure 43 were divided into 48-hour intervals and the average effluent nitrogen and volatile solids calculated for each interval. During this 19-day period, an average of 240 mg/l volatile solids was discharged in the effluent of the pond. From this figure, the actual production of algae per liter of influent can be calculated from evaporation data by assuming that one-fourth of the influent was lost through evaporation. Based on this calculation, about 180 grams of volatile material were shown to be produced from each liter of influent. If it is further assumed that algae are about 90 percent volatile, then the actual algal production was approximately 200 mg/l.

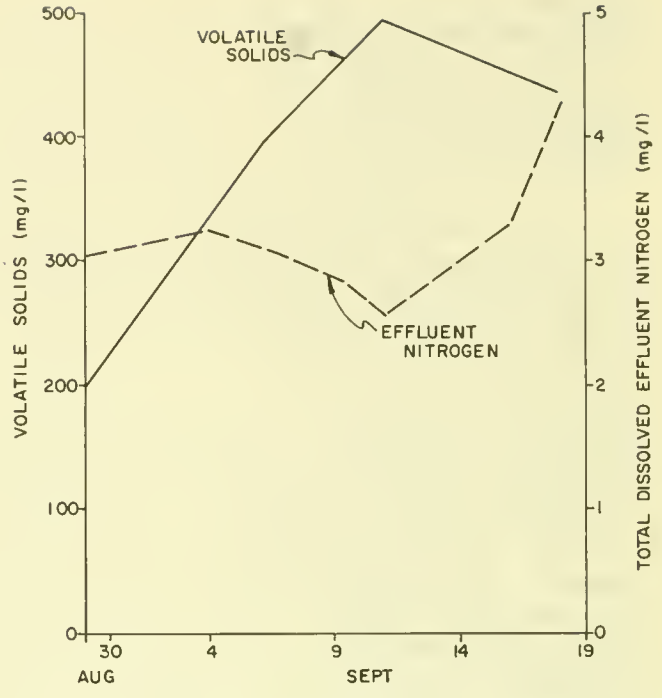


FIGURE 43-EFFLUENT NITROGEN AND VOLATILE SOLIDS USED IN BIOMASS PRODUCTION FIGURES

For the 19-day period, an average of approximately 17 mg/l of influent nitrate as N was used in the production of the biomass. Chemical analyses showed that the volatile solids were about 8.3 percent nitrogen. These data result in the following nitrogen balance:

$$C_I \text{ should} = C_{DE} + C_{AE} - (C_{Ai} - C_{Ae}) + C_x$$

$$185 \text{ should} = 23 + 146 + 6 + C_x$$

$$185 = 175 + C_x$$

where C_I = grams N entering system

C_{DE} = grams N dissolved in effluent

C_{AE} = grams N in effluent algae

C_{Ai} = grams N in in-pond algae-initial

C_{Ae} = grams N in in-pond algae-final

C_x = grams N not accounted for

Although an exact balance was not obtained, the difference, C_x, could be in the range of experimental and sampling error, or could be a measure of nitrogen lost by some mechanism such as denitrification. Based on the information accumulated thus far, it is estimated that the overall biomass yield for the algal system will be on the order of 1 ton of volatile solids per million gallons of waste plus 1/2 ton of inert materials.

Algal Genera Noted. The number of algal genera which were noted in the pond cultures was relatively low. The only genera that dominated the cultures are shown in Table 10. As previously noted Scenedesmus quadricauda was originally introduced, but occasionally Scenedesmus dimorpha was noted although never as the dominant algae. Scenedesmus was typically in the two- or four-celled stage, although in very active cultures the alga took a unicellular form with four small spines. The soil ponds went through several algal successions, with some flagellated algae (usually Carteria, Phacus, or Euglena) as the dominant genera. The soil ponds also often contained large numbers of an extremely small unicellular green algae, either Chlorella or Nannochloris.

TABLE 10

DOMINANT ALGAL GENERA AT THE IAWTC

Genus	Type
Scenedesmus	green
Oscillatoria	blue-green
Dysmorphococcus	green
Schroederia	green
Lagerheimia	green
Navicula	diatom

Scenedesmus was originally seeded into the RGP in the spring of 1968 and dominated the cultures until early summer of that year. At that time, species of other genera, mainly of Dysmorphococcus and Oscillatoria, began to dominate the cultures and, by August, Scenedesmus had almost entirely

disappeared. The RGP was then drained, cleaned, and refilled, and another 100 pounds of Scenedesmus sp. obtained from the Richmond Field Station were added. From then on, Scenedesmus was the dominant species in the RGP, although the culture was killed by CO_2 in the summer of 1969 and was reseeded from the miniponds. Summer conditions appeared to be particularly suitable for growth of the blue-green alga, Oscillatoria. During both 1968 and 1969, this alga was prevalent in some minipond cultures. Scenedesmus reestablished dominance with the onset of cooler weather.

All of the algae listed in Table 10, with the exception of diatoms and Lagerheimia, appeared capable of removing large quantities of nitrate-nitrogen. The appearance of large numbers of diatoms or Lagerheimia was usually accompanied by a decrease in nitrogen assimilation.

Predatory Organisms. Relatively little emphasis was placed on this aspect of pond ecology. However, during the spring and early summer of 1969, some data were collected on potential algal predators. Rotifers were by far the most common of such organisms with populations of 30,000 organisms/liter noted on occasion. In ponds with actively growing cultures of algae, rotifers were always present in numbers fewer than 500/liter. Large numbers of rotifers usually were found in ponds with senescent or dying algal populations, but it is not possible to state whether rotifers preceded or followed the senescent conditions.

Planktonic crustaceans (Copepoda, Daphnia, etc.) were notably absent from the algal ponds, and only in the two soil ponds were these organisms prevalent. Although usually fewer than 1,000 Copepod nauplii and adults per liter appeared in the cultures, on one occasion more than 5,000 of these organisms per liter were noted in a soil pond.

Dissolved Effluent Nitrogen. In general, the dissolved effluent nitrogen contained only nitrate, nitrite, and organic nitrogen. Ammonia, if detectable, was almost always less than 0.1 mg/l, compared to an undetectable amount occurring in the influent. Biological activity in the miniponds did cause dissolved organic nitrogen to increase from about 0.4 mg/l to 0.7 - 0.8 mg/l. Dissolved organic nitrogen usually was higher in the soil ponds than in the remaining miniponds. In minipond run 8 conducted during August and September 1969, dissolved organic nitrogen in all the miniponds was consistently above 1 mg/l and occasionally greater than 1.5 mg/l.

The concentration of nitrite-nitrogen followed a consistent pattern in the outdoor growth studies. After the ponds were filled, nitrite generally increased to 1 to 2 mg/l

and then, as the nitrate decreased, nitrite followed the same trend. This pattern is illustrated in Figure 44, which shows a minipond with a high percentage of total nitrogen removal. The ponds with low effluent nitrate usually also had a low nitrite concentration.

On several occasions, effluent nitrate-nitrogen concentrations were determined at two- or three-hour intervals for a 36-hour period. The variations in concentration were beyond the limits of the accuracy of the analysis; thus, a diurnal change in the concentration of this constituent could not be demonstrated.

Dissolved Oxygen. This parameter was not routinely monitored, although determination of dissolved oxygen concentration was a part of most diurnal studies. An example of the diurnal fluctuations in dissolved oxygen in four miniponds (data from September 10-11, 1968) are shown in Figure 45. Ponds 1, 4, and 22 each showed approximately 75 percent total nitrogen assimilation, whereas pond 10 showed only about 40 percent. The greatest fluctuation was noted in pond 22, an unmixed soil pond. Undoubtedly this change occurred because the afternoon mixing tended to deoxygenate the pond water in the remaining ponds. The 5 mg/l minima noted in ponds 1, 4, and 22 were near the minimum concentrations recorded in any of the overnight studies.

It is interesting to note that the deoxygenation factor for the ponds is on the order of 1.25 mg/l/hr, whereas the oxygenation factor is a net of 2.5 mg/l/hr, or a gross of

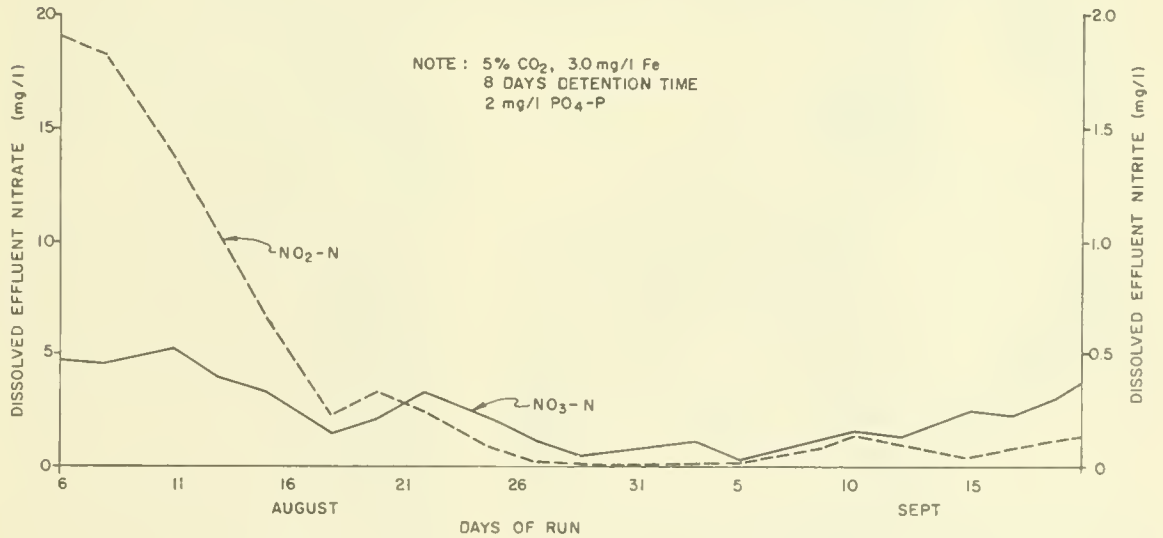


FIGURE 44-CHANGE IN NITROGEN FORMS DURING MINIPOND RUN

TABLE 11

EFFECT OF CARBON DIOXIDE AND AERATION
ON ALKALINITY IN MINIPOND RUN 8

Pond No.	Addition	Detention time (Days)	8/12/69		8/26/69		9/5/69	
			pH	Alkalinity+	pH	Alkalinity+	pH	Alkalinity+
12	100% CO_2	5	9.3	185	7.0	529	7.75	500
4	5% CO_2	5	9.25	164	7.9	428	7.8	369
20	Control	5	9.2	168	8.8	297	8.6	305
RGP	No air	10	9.2	197	8.9	280	8.75	235
Influent			7.6	374	7.5	373	7.5	373

+ mg/l as $CaCO_3$

about 4 mg/l/hr. If an oxygen : algae ratio of 1.5 is assumed, the rate of algae growth is 2.66 mg/l/hr, or about 32 mg/l/12 hr-day. At this rate about eight days would be required in September to attain the 240 mg/l algae required for 90 percent N assimilation. This detention agrees with the required detention times illustrated in Figure 29.

pH and Alkalinity. As noted earlier, growth of algae usually affects the pH and alkalinity of systems exposed to the atmosphere. A typical minipond without CO_2 addition would reach an afternoon pH of more than 10 and bicarbonate alkalinity approaching zero, as calculated by the table in Standard Methods. During this period of low bicarbonate alkalinity, algal growth probably was carbon limited. Some miniponds also showed a diurnal change in total alkalinity caused by precipitation and redissolving of calcium carbonate. The precipitation caused the pond surfaces to collect a scale, which was made up of a complex of phosphorus, magnesium, and calcium carbonates. Alkalinity samples had to be filtered to eliminate a sliding end-point caused by the presence of colloidal calcium carbonate in the sample.

As shown in Figure 46, the addition of 100 percent CO_2 had the effect of providing the algae with bicarbonate alkalinity. In ponds to which CO_2 was added, the diurnal pH change was minimized and the pH usually never reached more than 8.5 to 9.0; bicarbonate alkalinity was also present at all times of the day. A further effect of added CO_2 is shown in Table 11. With the lowering of the pH level brought about by the CO_2, the calcium carbonate scale went back into solution, which in turn raised the total alkalinity. In the example shown, there was greater than 100 mg/l (as $CaCO_3$) more effluent alkalinity by the end of the run than in the influent. This particular minipond run was started with a culture which had not received CO_2 previously; thus, the initial pond alkalinities had been lowered by $CaCO_3$ precipitation. The data for pond 20, the control pond, indicates that the cultures were not very active (low pH and alkalinity), and this observation is verified by the nitrogen removal data. Only about 50 percent of the total influent nitrogen was removed in this minipond.

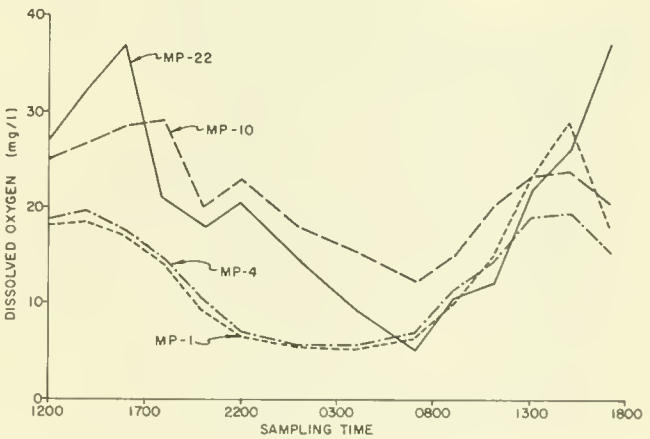


FIGURE 45-DIURNAL FLUCTUATIONS IN DISSOLVED OXYGEN

RGP Evaporation and Temperature Data. A weather station located in the rapid growth pond provided information on daily rates of evaporation loss and temperature change during 1969. Evaporation data was used to obtain mass balances of water and dissolved nutrients passing through the pond.

The RGP, filled to a depth of 12 inches, contained 81,000 gallons of water, or slightly less than 7,000 gallons per inch of depth. Figure 47 shows that the minimum daily evaporation loss was nearly -0.06 inch, which occurred in February 1969 (an atypically rainy month) and the maximum daily evaporation loss was 0.4 inch in August. An evaporation of 0.4 inch of pond water represents a volume loss of about 2,800 gallons. Total evaporation loss for the entire year was about 67 inches of water.

Detention times during the period of maximum evaporation were about 8 days, which required an influent flow of about 10,000 gallons of drainage water per day. Because of evaporative losses of 2,800 gallons per day, the effluent flow was only about 70 percent of the influent. The numbers shown for nitrogen removal were based on effluent concentrations compared to

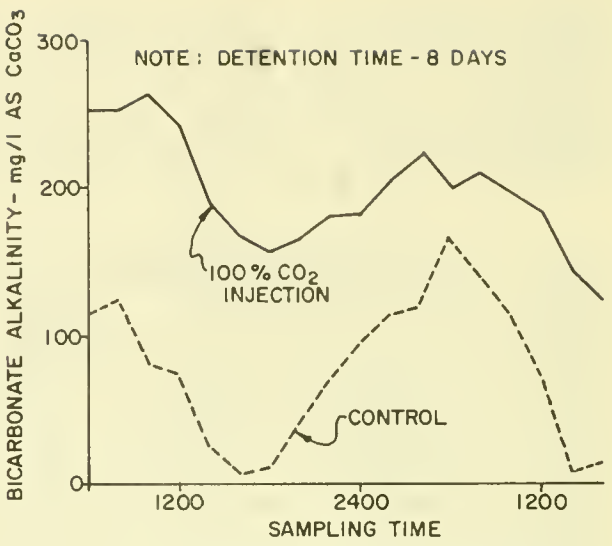


FIGURE 46- DIURNAL CHANGES IN BICARBONATE ALKALINITY

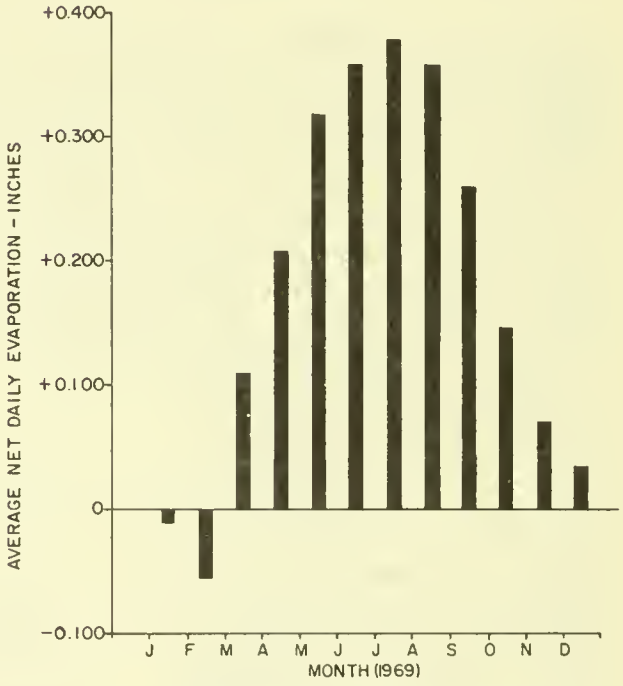


FIGURE 47- AVERAGE DAILY EVAPORATION IN RAPID GROWTH POND DURING 1969

influent concentrations, without regard to total pounds of nitrogen entering and leaving the system. This type of measurement was used because an effluent standard of 2 mg/l total dissolved nitrogen was in the original study objective. Computation of percent assimilation based on weights of nitrogen in the influent and effluent would yield higher rates of removal efficiency, especially during periods of maximum evaporation.

Presented in Figure 48 are the daily maximum and minimum pond temperatures observed in the RGP during 1969. During the months of May through early September, the maximum pond temperatures were consistently in the 80 to 90°F (27 to 32°C) range. The highest water temperature recorded during the entire period was 92°F (33°C) -- about 6 to 8°F (4°C) cooler than the maximum minipond temperature. The minimum temperature was 38°F (3°C) in late December. About September 15 there was a sudden 9 to 10°F (6°C) decrease in temperature from about 85 to 87°F (about 30°C) down to 75 to 78°F (about 24°C). Although temperatures were still quite warm, a decline in nitrogen assimilation accompanied the drop in almost all of the minipond growth units. Examination of the RGP nitrogen removal as graphed in Figure 39, shows that a similar decline in nitrogen uptake occurred; and, as the pond warmed up, the nitrogen removal was resumed at its former rate. This example was included to indicate that shallow algal cultures respond to changes in temperature.

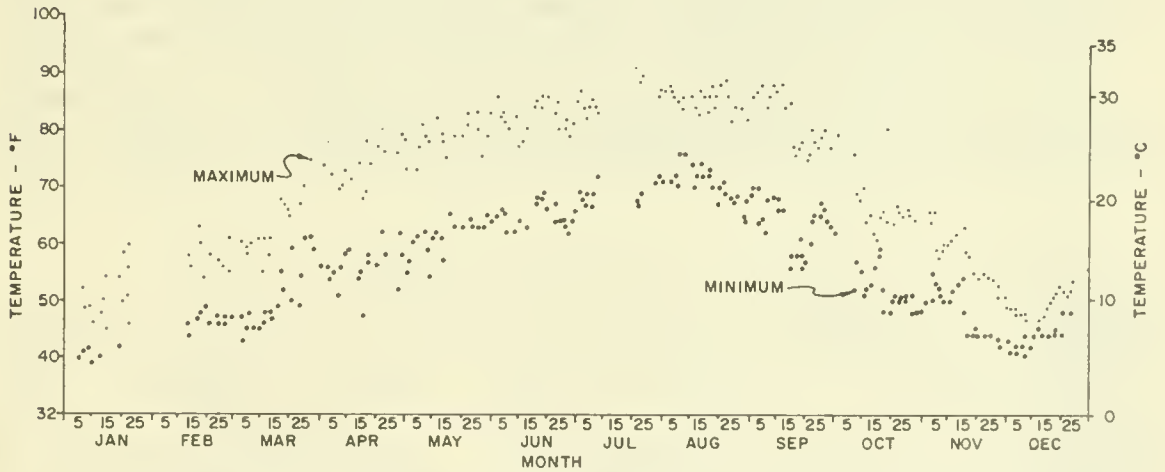


FIGURE 48 - DAILY MAXIMUM AND MINIMUM TEMPERATURES IN RAPID GROWTH POND DURING 1969

Laboratory

Lime, alum, and ferric sulfate were primary coagulants evaluated during the studies on harvesting of algae. The concentration required of each of these chemicals to remove the desired amount of algae varied according to the operating conditions of the growth units and was relatively independent of the algal concentration. The range in concentrations is illustrated in Figure 49 in which is shown the amount of each reagent needed to remove at least 90 percent of algae from rapid growth pond samples. Although all three chemicals proved to be effective coagulation aids, each had distinctive characteristics which added to or detracted from its usefulness.

The concentration of lime needed for the desired level of removal varied from 20 to 200 mg/l added as a 16,000 to 32,000 mg/l slurry; however, during normal pond operation (growth at or near the optimum level for maximum nitrogen assimilation) the required concentration was 20 to 40 mg/l. Calcium hydroxide raises the pH of a solution and causes algal flocculation by precipitation of mineral salts at elevated pH values; hence any addition to the growth unit that lowers pH will tend to increase the amount of lime needed for flocculation. A comparison of two miniponds, one receiving CO_2 (pH of 8.0) and one not receiving CO_2 (pH of 9.8) showed that the lime requirement was increased tenfold by the addition of carbon dioxide. Overnight studies involving the use of lime as a flocculent indicated that little diurnal change occurred in chemical requirements, although the best separation with lime took place in the afternoon when pond pH was maximum.

The high pH values associated with the addition of lime result in the precipitation of potential inorganic algal

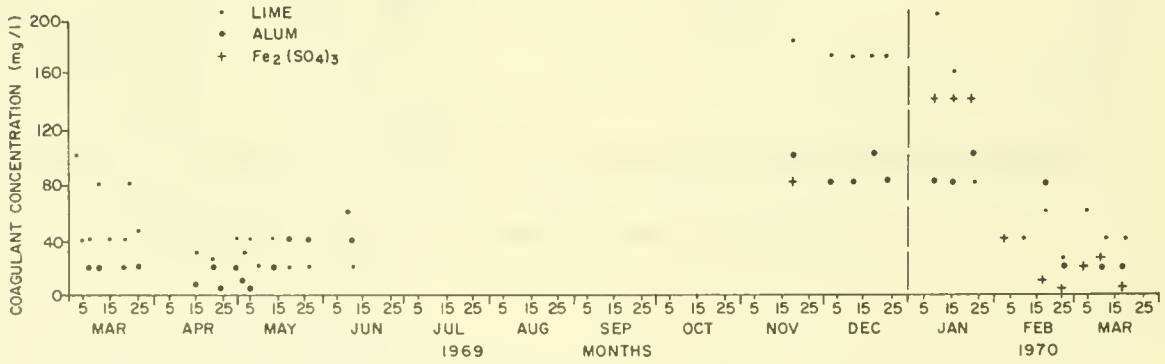


FIGURE 49 - CONCENTRATIONS OF MINERAL COAGULANTS REQUIRED FOR AT LEAST 90% ALGAL REMOVAL, JAR TESTS

nutrients, particularly phosphorus. Analysis of water samples before and after algal separation showed almost complete phosphorous removal, as well as removal of about one-half of the original magnesium and total alkalinity. Although total hardness was reduced by one-third, the calcium concentration was lowered only slightly, presumably because the calcium carbonate precipitate remained in the supernatant as a colloidal suspension. The pH levels of a lime-treated effluent may also be potentially detrimental in receiving waters because most discharge requirements call for pH levels in the 6.5 to 8.5 range. If such discharge requirements are set for the plant effluent, then pH adjustment by aeration, acid addition, etc., will be necessary. Some very preliminary work on the time required for reaching a pH equilibrium by atmospheric exchange indicates that it will be about two to three days. If this time interval is adequate, the pH of the treated drainage water would probably reach an acceptable range during transit from the treatment plant to the Delta discharge point.

Aluminum sulfate, the second compound, causes coagulation by the action of simple metal ions, e.g. $(AlH_2O)_6^{+3}$, and by the formation of hydrolysis products which may be highly charged, multinuclear, hydroxometal complexes (Stumm and Morgan, 1962). Alum is normally more effective at low pH levels. However, at Firebaugh the results indicated that the cost of adjusting the pH to the optimum level was generally higher than the cost of the additional coagulant required to achieve the same flocculation. Some preliminary work also indicated that pH adjustment plus rapid mixing resulted in release of soluble cellular nitrogen, thus lowering the overall efficiency of the nitrogen removal process. Alum concentrations to bring about 90 percent removal of algae ranged from 5 to 200 mg/l, with 20 mg/l necessary when the pond was operated at the best conditions for nitrogen assimilation. Although pH values were lower when carbon dioxide was added, alum requirements were slightly higher with than without the CO_2. Alum flocculation removed dissolved phosphorus but was not accompanied by a change in pH to an unacceptable level.

The third compound receiving extensive testing was analytical grade ferric sulfate. Its effective concentration ranged from 1 mg/l to about 140 mg/l (as $Fe_2(SO_4)_3$), depending mainly on the presence of ferric chloride in the rapid growth pond. As stated earlier, ferric chloride, 3 to 6 mg/l, was added to the growth unit to enhance algal growth. It can also be used as a coagulant. The dramatic effect of the addition of iron to the RGP on required dosages of all three chemicals is illustrated in Figure 50. Without any additional chemical the removal level was increased from 40 to 70 percent, presumably as a result of coagulation by the ferric chloride added for growth. The effective level of $Fe_2(SO_4)_3$ addition

was reduced from 140 mg/l to 5 mg/l. The concentrations of both lime and alum were also reduced, with alum being effective at about 20 mg/l and lime at 40 mg/l. The effect of iron added to the RGP in terms of reduction in required dosage for coagulation can also be seen in Figure 49. Although only 1 mg/l was added to the pond daily, the concentration of total iron in the pond tended to increase gradually. The growth studies indicated that as a necessary addition to the algal ponds iron will provide a dual benefit.

Shown in Table 12 is the effect of adding lime, alum, and iron on the cost of removing 90 percent of the algae from one million gallons of plant effluent. (This does not include the cost of the $FeCl_3$ needed for algal growth.) Figures in the table show that the cost of these chemicals for separation of algae may be extremely low and that ferric sulfate was the cheapest of the primary flocculents tested. Additions of 1 to 5 mg/l of this compound would probably have little effect on the algae's potential market value.

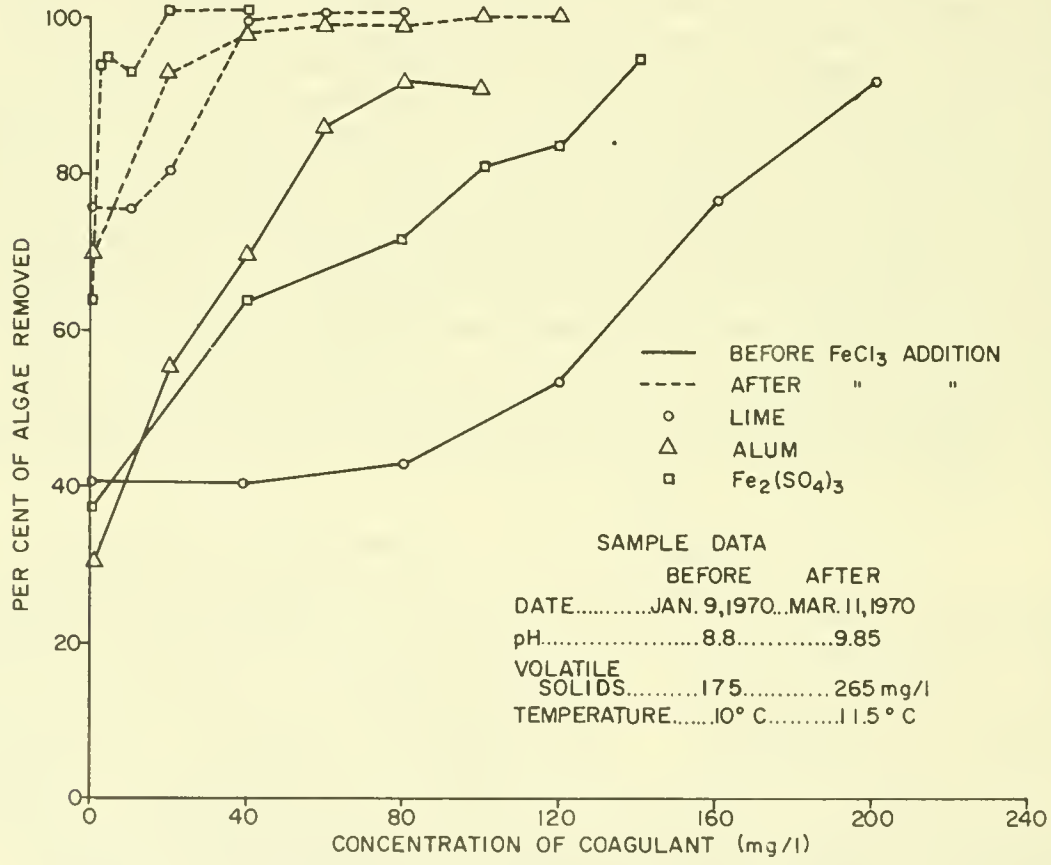


FIGURE 50-CONCENTRATIONS OF LIME, ALUM AND FERRIC SULPHATE REQUIRED FOR 90% ALGAE REMOVAL - BEFORE AND AFTER ADDITION OF FERRIC CHLORIDE TO RAPID GROWTH POND

In addition to the mineral coagulants, approximately 60 polyelectrolytes were tested for their effectiveness as primary coagulants, or in combination with lime, alum, or ferric sulfate. A list of the common names of these compounds, along with data on their effectiveness, either alone or in combination with a mineral coagulant, is shown in Table 13. Of the 60 polyelectrolytes, the 17 compounds listed in Table 14 were found to be effective coagulants and their costs were economically competitive with mineral coagulants alone. In general, required concentrations of polyelectrolytes less than 10 mg/l effected coagulation. If iron was added to the growth pond, the effective concentration was often as low as 0.05 to 0.25 mg/l. The list of polyelectrolytes in Table 13 includes some compounds that were evaluated when iron was not added to the pond, and the results may be somewhat misleading in that iron addition affects the performance of the polyelectrolytes. Many of these polyelectrolytes will be tested again during calendar year 1970 to determine whether their effectiveness is influenced by the addition of iron to the growth units. One compound, Cat-Floc (Calgon Corp.) was not beneficial in early testing, but with the addition of iron to the RGP, more than 90 percent of the algae could be removed with 0.5 mg/l of this compound. The cost of treating one million gallons of algal pond effluent at this concentration of Cat-Floc would be about $2.00.

TABLE 12

ESTIMATED COST OF LIME, ALUM, AND FERRIC SULFATE TO REMOVE 90 PERCENT TOTAL SUSPENDED SOLIDS (Dollars/million gallons)

Date	Volatile Solids (mg/l)	Alum	Lime	Ferric Sulfate
11/19/69	250	11.30	16.80	14.40
12/03/69	200	12.30	14.60	
12/11/69	100	14.00	20.00	25.00
1/09/70	175	13.30	20.80	23.80
1/16/70	71	11.90	15.90	22.30
1/23/70	91	12.70	8.20	24.40
2/11/70*	114		3.50	11.00
2/18/70	210	13.00	5.40	1.50
2/25/70	417	2.35	1.30	0.25
3/11/70	265	3.46	3.28	0.40
3/18/70	229	3.50	3.23	0.62

*Commenced 1 mg/l daily addition of Fe^{+++} to pond on 1/26/70.

TABLE 13

RESULTS OF LABORATORY TESTS OF THE EFFECT OF VARIOUS POLYELECTROLYTES ON FLOCCULATION AND SEDIMENTATION OF ALGAL CULTURES AT THE IAWTC

Compound	Company	Effect on Flocculation[1] Alone	With Alum	With Lime	Tested Conc. Range (mg/l)
Cat-Floc	Calgon Corp.	B	B	B	1-10
ST-260	Calgon Corp.	N	N	N	0.25- 4
ST-266	Calgon Corp.	N	N	N	1-10
ST-269	Calgon Corp.	N	N	N	0.25- 4
ST-270	Calgon Corp.	N	N	N	0.25- 4
Drewfloc-3	Drew. Chem. Corp.	N	N	N	0.25- 5
Hercofloc 810	Hercules, Inc.	B	B	B	0.25-10
Hercofloc 814	Hercules, Inc.	B	B	B	0.25-10
Kelgin W	Kelco Company	N	N	N	0.25-10
Kelcosol	Kelco Company	N	N	N	0.25-10
Kelzan	Kelco Company	N	N	N	0.25-10
Kelco SCS MV	Kelco Company	SB	SB	SB	0.25-10
Kelco SCS HV	Kelco Company	SB	SB	SB	0.25-10
Ferrifloc[2]	Tennessee Corp.	B			5-60
Chitosan	Chem. Res. Lab, Inc.	N	N	N	1-10
Gendriv 162	General Mills, Inc.	B	N	B	1-10
Genfloc 139	General Mills, Inc.	N	N	N	0.5 - 5
Genfloc 140	General Mills, Inc.	N	N	N	0.5 - 5
Genfloc 155	General Mills, Inc.	B	B	B	0.5 - 5
Genfloc 156	General Mills, Inc.	B	B	B	0.5 - 5
Genfloc 292	General Mills, Inc.	N	B	B	0.06- 2
Polyhall M-293	Stein-Hall	N	N	N	0.25-10
Polyhall M-402	Stein-Hall	N	N	N	0.25-10
Polyhall M-603	Stein-Hall	N	N	N	0.25-10
Jaguar MDD	Stein-Hall	N	N	N	0.25-10
Jaguar MAL-22-A	Stein-Hall	N	N	N	0.25-10
Primafloc C-3	Rohm and Haas	B	B	B	1-10
Primafloc C-5	Rohm and Haas	B	B	B	1-10
Primafloc C-7	Rohm and Haas	B	B	B	0.5 - 5
Zeta floc WN-2	Narvon Mines, Ltd.	N	N	N	5-80
Zeta floc C	Narvon Mines, Ltd.	N	N	N	5-80
Zeta floc O	Narvon Mines, Ltd.	N	N	N	5-80

TABLE 13 (Continued)

Compound	Company	Effect on Flocculation[1] Alone	With Alum	With Lime	Tested Conc. Range (mg/l)
Nalcolyte 603	Nalco Chemical Co.	N	B	N	1-10
Nalcolyte 610	Nalco Chemical Co.	N	N	B	1-10
PEI 6	Dow Chemical Co.	N	N	B	0.5 - 5
PEI 12	Dow Chemical Co.	N	N	B	0.5 - 5
PEI 18	Dow Chemical Co.	N	N	B	0.5 - 5
PEI 600	Dow Chemical Co.	B	B	N	1.0 -10
SA 1188-1A	Dow Chemical Co.	N	N	N	1.0 -10
SA 1188-1F	Dow Chemical Co.	N	N	N	1.0 -10
SA 1569	Dow Chemical Co.	N	B	N	1.0 -10
NC 1621	Dow Chemical Co.	N	B	N	1.0 -10
Purifloc A-21	Dow Chemical Co.	B	B	N	0.5 - 5
Purifloc A-22	Dow Chemical Co.	B	B	N	0.5 - 5
Purifloc C-31	Dow Chemical Co.	B	B	N	1-10
Purifloc C-32	Dow Chemical Co.	B	B	N	0.5 - 7
Purifloc NP-10	Dow Chemical Co.	B	B	N	0.5 - 5
Wisprofloc-20[3]	Ionae Chemical Co.	N	N	N	1-10
Wisprofloc-P	Ionae Chemical Co.	N	N	N	1-10
Burtonite #78	Burtonite Co.	N	N	N	1-10
Magnifloc 530C	Amer. Cyanamid	N	N	N	0.06- 2
Magnifloc 837A	Amer. Cyanamid	N	N	N	0.5 - 5
Magnifloc 521C	Amer. Cyanamid	B	B	B	0.06- 2
Magnifloc 820A	Amer. Cyanamid	B	N	B	0.06- 2
Magnifloc 835A	Amer. Cyanamid	B	N	B	0.06- 2
Magnifloc 836A	Amer. Cyanamid	B	N	B	0.06- 2
Magnifloc 860A	Amer. Cyanamid	N	N	N	0.06- 2
Magnifloc 870A	Amer. Cyanamid	N	N	N	0.25- 4
Magnifloc 900N	Amer. Cyanamid	N	N	N	0.06- 2
Magnifloc 905N	Amer. Cyanamid	N	N	N	0.06- 2
Magnifloc 985N	Amer. Cyanamid	N	N	N	0.06- 2
Magnifloc 990N	Amer. Cyanamid	N	N	N	0.06- 2

1/ The effect has been denoted as N - no help; B - beneficial; and SB - slightly beneficial. A beneficial effect was shown if the polyelectrolyte aided flocculation and sedimentation with no consideration to the economic aspects of its use.

2/ Not a true polyelectrolyte, but a crude iron sulfate ore.

3/ Not commercially available in this country.

TABLE 14

POLYELECTROLYTES WHICH WERE EXPERIMENTALLY BENEFICIAL AND ECONOMICALLY FEASIBLE AIDS IN ALGAL SEPARATION

Manufacturer	Name	Effective Concentration Range (mg/l)			Primary Coagulant		
		1/	2/	3/	None	Aluminum Sulfate	Calcium Hydroxide
Dow Chemical Co.	PEI 600	5-10	0.5-4.0		X	X	
Dow Chemical Co.	C-31	0.5-5	0.25-4.0		X	X	
Dow Chemical Co.	C-32	0.5-5	0.5-1.0		X	X	
Nalco	Nalcolyte 603		4-8			X	
Nalco	Nalcolyte 610			4-6			X
Rohm and Haas	Primafloc C-3		4-8			X	
Rohm and Haas	Primafloc C-5	4-8	0.25-4.0		X	X	
Rohm and Haas	Primafloc C-7	5-10	0.25-1.0		X	X	
Hercules, Inc.	Hercofloc 814	1-3	0.5-1.0	0.5-1.0	X	X	X
General Mills, Inc.	Glenfloc 155		0.25-1.0	0.25-1.0	X	X	X
General Mills, Inc.	Glenfloc 156		0.25-1.0	0.25-1.0	X	X	X
General Mills, Inc.	Glenfloc 162		0.25-1.0	0.25-1.0	X	X	X
American Cyanamid Co.	Magnifloc 521C*		0.25-1.0	0.25-1.0	X	X	X
American Cyanamid Co.	Magnifloc 820A**			0.25-1.0			X
American Cyanamid Co.	Magnifloc 835A**			1-2			X
American Cyanamid Co.	Magnifloc 836A**			1-2			X
Calgon Corp.	Cat-Floc	0.05-0.4	0.05-1.0		X	X	X

1/ Alone

2/ With alum

3/ With lime

*C - cationic

**A - anionic

Polyelectrolytes reduce the zeta potential, a term involving the net particle charge and the distance the charge affects the surrounding medium (Fair, et al, 1968), thus allowing colloids to approach each other (Black, et al, 1965). The polymer molecules also aid in the formation of three dimensional algal-polymer matrices by attaching themselves to the surface of algal cells (Tenney, et al, 1969). Algae generally have a net negative surface charge (Ives, 1956). Tenney, et al (1969) found that only cationic (positively charged) polyelectrolytes effected algal flocculation. At Firebaugh cationic polymers were generally more effective; however, three anionic compounds (Magnifloc 820A, 835A, and 836A) did cause coagulation and floc formation when used with lime. Tenney, et al, (1969) also noted that polymer effectiveness was affected by pH (more effective at lower pH ranges), algal concentration, and algal growth phases. Their observation that there was an optimum polyelectrolyte concentration was not demonstrated at the IAWTC; however, the range of tested concentrations was relatively narrow, and we may have missed the decline at higher levels of polymer since we tried to stay within an economic range.

The use of polyelectrolytes to flocculate algal suspension was often the most economical means of achieving algal removal. However, their use may be limited if the algal product is to be used as an animal food. Studies need to be conducted to determine whether cationic polyelectrolytes are toxic to animals. Preliminary tests run by Golueke, et al (1964), in which the cationic polyelectrolyte Sondellite was used, showed that single massive dosages of the compound had no demonstrable effect on weanling rats. Daily dosage at a level comparable to that which the animals would receive from Sondellite-flocculated algae resulted in no apparent symptoms for a period of two weeks, the duration of the test. Another possible problem which might arise through the use of polyelectrolytes would be the rise in effluent dissolved nitrogen caused by the nitrogen in the polyacrylamide and polyamide compounds. At the effective concentration used at Firebaugh. this problem should not be significant.

Pilot Scale Separation Studies

Sedimentation Tank. This unit was not evaluated as a separation device per se, but was used to control biomass levels in the rapid growth pond. Water from the RGP was pumped into the unit at flows which gave theoretical hydraulic detention times in the tank of two to six hours. The algal sludge from the unit ranged up to 3 to 5 percent solids, depending on retention time of the solids. The sun-dried algal product from this unit provided a

source of algae for companies interested in studying the market potential of the product. From March 10 through March 14, 1970, a total run time of 95 hours of sedimentation in this unit resulted in the removal of about 50 pounds (22.7 Kg) of suspended solids from the RGP. Detention time, within the range of two to six hours, did not have any apparent effect on sedimentation.

The composition of the sedimentation tank effluent may be more significant than the actual production of an algal product. The suspended solids in the influent and algal sludge were about 50 to 70 percent volatile material (algae); whereas the effluent suspended solids were often as high as 90 percent volatile and consisted of the younger, actively growing Scenedesmus cells. The tank thus helped in the removal of the older and heavier cells, as well as the suspended chemical precipitates and clay particles from the growth pond.

Shallow Depth Sedimentation Tank (Water Boy). The shallow depth sedimentation tank was operated on-line with the rapid growth pond in a system in which growth pond effluent passed through the unit and then was discharged from the site. The algal slurry was used in the testing of the effectiveness of various dewatering devices. As described previously, separation was effected by coagulation-flocculation, with subsequent filtration of supernatant to remove cells not trapped in the algal floc.

The percent transmittance of light through effluent and influent samples over a three-month period is shown in Figure 51. The influent varied from about 150 to 700 mg/l total suspended solids. After the first of February 1970, the the growth pond was swept daily; thereafter, the suspended solids concentration in the influent to the sedimentation tank were consistently in the 300 to 500 mg/l range. During this period of the study the effluent from the Water Boy (after the mixed-media filter) usually contained less than 10 mg/l volatile solids (less than 20 mg/l total solids). Influent flows varied from 1.4 to 3 gpm with resulting hydraulic detention times of 93 to 44 minutes. At the flow rates tested, removal of algae was relatively independent of influent solids in the range of 150 to 700 mg/l.

The effluent data shown in Figure 51 were products of both sedimentation and filtration. Samples of the effluent of the sedimentation chamber indicated that more than 90 percent of the suspended solids were removed by coagulation-sedimentation and that the filter acted as a final polishing device. The mixed-media filter remained in the Water Boy until May 12, 1970, at which time the entire filter was removed because of plugging of the pores by what appeared to be

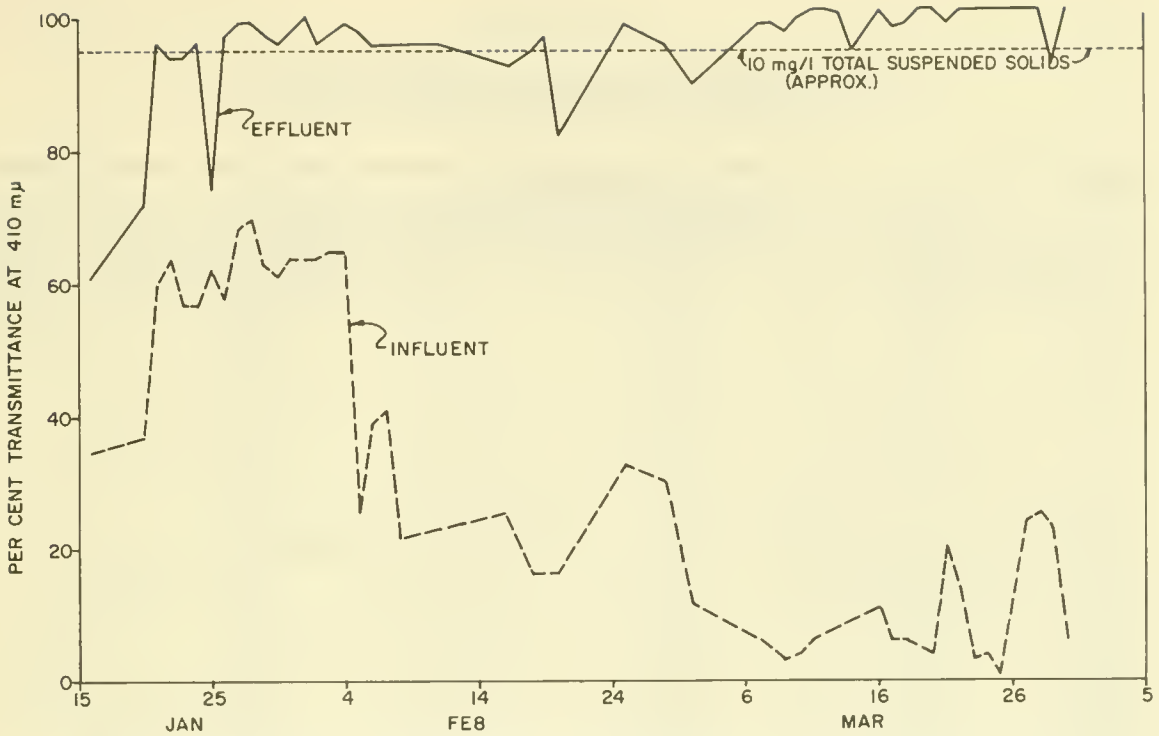


FIGURE 51-PER CENT TRANSMISSIONS OF WATER BOY INFLUENT AND EFFLUENT SAMPLES DURING FIRST THREE MONTHS OF 1970

chemical solids and chemical additives during this period of the study. Before the filter was removed, the unit was removing almost 100 percent of the influent solids. (The effluent concentration probably was greater than zero, but it could not be measured by the available analytical techniques.) After the filter had been removed, the percentage removal decreased by about 1 to 2 percent with as much as 27 mg/l total solids found in the effluent (average of 14 mg/l total effluent solids). The data show that the mixed-media filter was not essential to the algae removal by the Water Boy.

Chemical additions to the Water Boy were based on the results of weekly jar tests involving the use of alum or Ferrifloc in combination with Calgon Cat-Floc. (Ferrifloc is a commercially available product containing about 60 percent $Fe_2(SO_4)_3$.) Prior to March 1, the dosage generally consisted of 20 to 40 mg/l alum plus 1 to 3 mg/l Cat-Floc. After March 1, the addition of iron to the RGP made it possible to decrease the coagulant requirement to less than 1 mg/l Cat-Floc, with no alum being necessary. Concentrations of Cat-Floc as low as 0.2 mg/l effected complete removal of up to 600 mg/l suspended solids. With continued addition of iron to the growth unit, $Fe_2(SO_4)_3$ became the most effective and economical of the coagulants tested. Table 16 shows a comparison between

TABLE 15

WATER BOY OPERATIONAL CRITERIA AND RESULTS FOR MAY 1970

Date of Sample	Flow Rate GPM	Chemicals $Fe_2(SO_4)_3$ mg/l	Chemicals Cat-Floc mg/l	Percent Removal	Total Solids in Eff. mg/l
1	2.0	3.0	1.5	100	0
2	2.5	2.5	0.6	100	0
3	2.5	3.0	0.7	100	0
4	2.5	3.0	0.6	100	0
5	2.5	3.5	0.5	100	0
6	3.0	3.5	0.6	100	0
7	3.0	3.5	0.4	100	0
8-12	- - - - Shut Down - Removed Sand Media - - -				
13	2.5	3.0	0.7	97	21
14-15	2.0	3.0	0.7	97	21
16	2.0	3.0	0.8	99	7
17	2.0	4.5	0.7	99	7
18	2.5	3.0	0.6	99	7
20	2.5	0.8	0.7	97	22
21	2.5	2.0	0.7	98	15
22	3.0	2.5	0.6	98	15
25	3.0	2.0	0.4	97	27
26	3.0	2.5	0.4	98	18
27	3.0	4.5	0.4	99	9
28	3.0	5.0	0.3	98	18
29-31	3.0	5.0	0.4	100	0

four different coagulants -- alum, lime, Ferrifloc, and Cat-Floc -- on two different days. On both days, ferric sulfate was the most economical of the compounds tested. In the test, the $Fe_2(SO_4)_3$ was derived from Ferrifloc, and the dosage was measured as pounds of total material (i.e., Ferrifloc) which included waters of hydration, other compounds, etc. Laboratory comparisons (jar tests) of analytical grade $Fe_2(SO_4)_3$ and Ferrifloc indicated that the latter material was the more effective of the two materials.

The Water Boy was routinely backwashed every 48 hours and the algal slurry was sampled for total suspended and volatile solids. Some of the results of these analyses are given in Table 17. The slurry taken directly from the tubes contained from 1.1 to 1.6 percent solids with 50 to 60 percent of the solids as volatile material. Overnight settling of the slurry increased the percent solids to 4 to 6 percent; however, tests of settling as a function of time

TABLE 16

COST COMPARISONS OF FOUR COAGULANTS
(Dollars per Million Gallons)

Date	Coagulant	Cost for 90% Removal	Cost for 40 mg/l Effluent: Total Suspended Solids	RGP Variables
5/20/70	$Al_2(SO_4)_3$	8.83	14.45	pH 9.45
	$Ca(OH)_2$	6.22	7.02	Temp. 15.5°C
	$Fe_2(SO_4)_3$	0.77	0.77	Total Solids 726 mg/l
	Cat-Floc	2.17	5.93	Settleable Solids 14.0 ml/l
5/27/70	$Al_2(SO_4)_3$	2.59	9.26	pH 9.35
	$Ca(OH)_2$	3.06	3.88	Temp. 17.1°C
	$Fe_2(SO_4)_3$	0.16	0.48	Total Solids 999 mg/l
	Cat-Floc	1.44	3.87	Settleable Solids 19.0 ml/l

TABLE 17

COMPOSITION OF SLURRY FROM WATER BOY

Date	Total Suspended Solids - mg/l	Volatile Solids mg/l	Percent Volatile
3/11/70	16,000+	8,400	53
3/12/70	44,600o	22,800	51
3/16/70	14,200+	6,800	48
3/17/70	56,000o	24,000	43
3/18/70	16,200+	7,600	47
3/19/70	47,700o	22,400	47
3/20/70	13,900+	8,600	62
3/21/70	42,300o	26,300	62
3/22/70	11,200+	6,400	57
3/23/70	33,500o	20,000	60
3/24/70	14,800+	8,600	58
3/25/70	37,900o	18,700	57

+ = Slurry directly from unit.
o = Slurry after settling for 24 hrs. and decanting off liquid.

indicated that 80 percent of the settling occurred in the first six to eight hours. The settled slurry normally was held in a collecting sump to provide material for the dewatering studies.

Upflow Clarifier. The upflow clarifier used detention times of about one hour to remove up to 95 to 100 percent of the suspended solids and produced a slurry containing 2 to 10 percent solids. The only chemical additive tested was sodium hydroxide, and the required concentration varied from 100 to 200 mg/l.

Although the unit did do an effective job of removing the algae, many operational problems arose which prevented long run times. The most serious of the problems was caused by the consolidation of the algal mat, which increased hydraulic pressure on the mat and caused sloughing of the algal floc. The algae were then carried over with the effluent. When this occurred, normal operation of the unit did not resume until the mat was built up again. Until this problem is solved, the upflow-clarifier type of separation device cannot be used as a reliable method of removing algae. The upflow clarifier will be remodeled in an attempt to eliminate plugging.

Upflow Sand Filter (Sanborn Filter). Preliminary studies on the Sanborn filter constructed by Bohna began in June 1969 with an algal suspension from the rapid growth pond as the test medium. Although the early studies showed that the filter could separate the algae from the liquid phase, some problems soon developed, especially those concerned with backwashing of the unit. In the first runs, the filter was plugged after relatively short run times, and the algal concentration in the backwash was low. The low concentration in the backwash may have been caused by too large a backwash volume and by the plugging due to biological fouling. Washing the sand bed with dilute sulfuric acid did not lead to an increase in run time. However, treatment with dilute hypochlorite solution did remove the fouling materials.

Personnel from Bohna assisted in testing the filter for four days in the fall of 1969. As shown in Table 18, at a flow rate of 1 gpm, the unit consistently removed more than 90 percent of the influent solids. The loading on the surface of the filter during this test period ranged from 107 to 595 mg/ft^2/min with an average of 364 mg/ft^2/min. The variation in influent concentration was the result of the mixing cycle used in the RGP. Run length was limited by the maximum design pressure of the unit and varied from 40 to 100 minutes,

TABLE 18

SUMMARY OF OPERATIONAL DATA FOR
SANBORN FILTER, 11/17-21/69

Run No.	Feed Concentration Suspended Solids, mg/l	Filtrate Concentration mg/l	Percent Removal Susp. Solids	Backwash Volume (Gal.)	Backwash Conc. Total Susp. Solids (mg/l)
11/18/69-1	156	3.7	97.8	2.1	6,500
11/18/69-2	113	0.5	99.6	1.7	7,200
11/18/69-3	549	1.4	99.8	2.4	11,600
11/18/69-4	556	2.8	99.5	2.5	17,700
11/18/69-5	572	11.9	98.0	3.0	13,700
11/19/69-1	142	1.7	98.8	1.9	6,360
11/19/69-2	565	0.7	99.9	2.5	11,100
11/19/69-3	395	14.5	96.5	3.8	6,680
11/19/69-4	629	1.7	99.8	4.25	5,330
11/20/69-1	138	1.6	98.6	2.7	4,880
11/20/69-2	456	0.2	99.6	2.6	4,330
11/20/69-3	548	0.5	99.9	1.8	10,700
11/20/69-4	615	2.4	99.62	2.4	10,600
11/20/69-5	293	15.9	95.2	3.3	5,780
11/21/69-1	214	19.0	92.0	3.2	4,260
11/21/69-2	214	16.2	93.0	1.8	5,230

depending on the solids concentration in the influent. The relationship between run time and solids concentration is shown in Figure 52. Above 400 mg/l suspended solids the run time was uniformly near 40 minutes. Routine operation of an algal stripping plant would probably be characterized by a more uniform solids concentration, and thereby a stabilization of the run time.

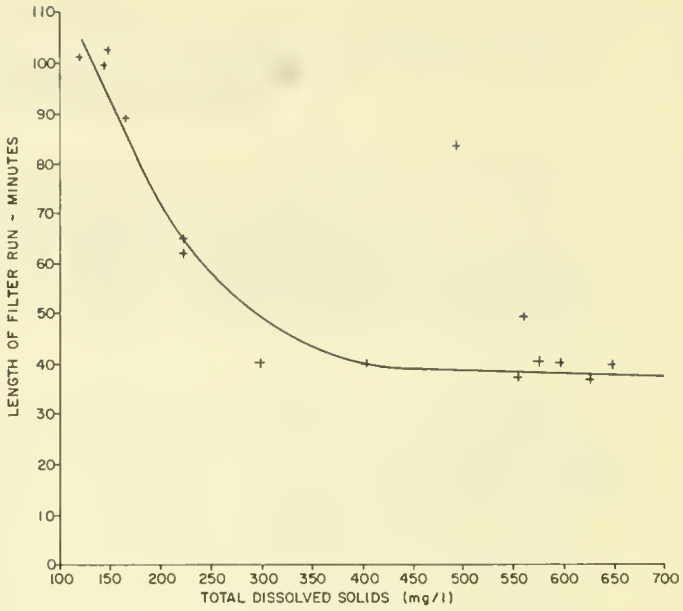


FIGURE 52- RELATION BETWEEN SOLIDS LOADING AND RUN LENGTH, SANBORN FILTER

The data in Table 18 also show the concentration of algae in the backwash. In this particular study, the unit was manually backwashed when the pressure differential across the filter reached 20 pounds per square inch (psi). Based on a one-run study of the effect of the backwash volume of two gallons (0.5 gallon/ft^2) was selected as the optimum quantity. Concentration of suspended solids in the backwash was roughly proportional to the initial feed concentration and varied from 0.4 to 1.7 percent solids. These concentrations could probably be increased to 3 to 5 percent with smaller backwash volumes and automated control of the backwashing cycle.

Another intensive five-day study of the Sanborn filter was conducted in March 1970, at which time flow rates of 1.0 and 1.25 gpm were tested. The one-gpm throughput used in the previous study corresponds to the manufacturer's design criterion of 0.25 gpm per square foot of filter surface. In this second run, the unit was operated on a continuous basis. The results were similar to those obtained in the November study with about 95 percent solids removal and backwash concentrations of 1.2 to 2.7 percent solids. After five days of operation, solids buildup within the unit appeared to cause a gradual decrease in run time and the buildup appeared to be significantly faster at the higher influent rate. Preliminary estimates showed that the expected filter life would be about 200 days at 1 gpm, compared to about 50 days at 1.25 gpm.

Vacuum Filter. Preliminary operation of the Eimco vacuum filter yielded disappointing results. No build-up of algal cake occurred on the filter belt, and the concentration of algae in the effluent was only slightly less than that in the influent. Microscopic examination of the algae and of the belt material indicated that the mesh openings in the belt were too large to retain the small (10 to 25 micron) algal cells. Based on these results, a multi-filament nylon belt (Eimco No. NY-319F) with a pore size similar to that of the algae was substituted for the existing porous polypropylene belt. The unit was then evaluated as a means of dewatering a concentrated algal slurry.

The first three-day run with the new belt was completed in January 1970. The feed concentration was about 3,000 mg/l (0.3 percent solids). Although this concentration of algae was considerably lower than the planned 3 percent solids from the concentration step, it did permit an estimation of the unit's effectiveness at lower algal loadings. The unit appeared to take up water at a fixed rate of about 1 gpm, resulting in a suspended solids loading on the belt of about 3,720 mg/ft^2/min. Only one belt speed, 2.9 ft/min (or 2.8 ft^2/min), was evaluated. During this test period, the unit produced sludge containing 18 to 25 percent solids and removed 90 to 95 percent of the influent suspension, with an average concentration of solids in the effluent of about 300 mg/l. The high removal rates were obtained with a vacuum of 15 to 20 inches of mercury (Hg). When the vacuum was lowered to 5 inches of Hg, less than 20 percent of the algal solids were removed (influent -- 3,880 mg/l; effluent -- 3,080 mg/l).

A second run was completed in which an algal slurry of about 30,000 mg/l (3 percent solids) was used. The resulting loading on the belt was 17,500 mg/ft^2/min. The vacuum in this run was 20 to 25 inches of Hg, or about 5 inches more than in the previous test. The unit removed about 90 percent of the influent solids and produced an algal product containing 24 percent solids. In both of these tests, effluent from the unit contained algal concentrations higher than allowable and would thus have to be recycled to the head of the sedimentation area.

Microscreen. The Zurn microscreen tested was supplied with screens of two pore sizes, 25 and 35 microns. Operation of the unit soon showed that algae were passing through the finer screen. Removals up to 30 percent were obtained, but most of this was due to algal settling in the influent and effluent chambers. A more complete evaluation

of this unit will require smaller pore-size screens, and perhaps testing of different pore sizes in series.

Centrifuges. Two of the centrifuges tested at Firebaugh, the continuous solid bowl type and the nozzle type, were not particularly efficient at either primary concentration or dewatering. With the solid bowl centrifuge, algal slurries with concentrations of 3 to 22 percent solids were obtained, but the highest removal rate was only 28 percent. A major difficulty with the nozzle-type centrifuge was the plugging of the nozzle openings and consequent failure of the machine to remove algae. The plugging did not appear to be caused by the algae but by colloidal clay particles in the water.

A third type of machine, the De Laval self-cleaning centrifuge, was evaluated as both a primary concentration and as a dewatering device. Used as a primary concentrator, the unit removed up to 95 percent of the influent (concentration, 800 mg/l). The flow through the unit was 6 gpm and the effluent slurry contained about 10 to 12 percent solids. Operation was continuous under these experimental conditions. A problem in plugging was encountered because of incomplete discharge of the material in the bowl. This problem was resolved through the use of a so-called "double shoot", in which the machine was programmed to partially discharge the contents followed by a complete ejection. Using this double-shoot concept, a product with up to 17 percent solids was obtained, but the percent removal dropped to the low 80's.

The self-cleaning centrifuge was also tested with an influent containing 20,000 to 30,000 mg/l influent solids. In this test the flow rate was 2.75 gpm, while the slurry contained about 10 percent solids. At this loading, the centrifuge removed more than 98 percent of the influent suspended solids.

Oswald and Golueke (1968) reported on the use of centrifugation to separate sewage-grown algae. With a 30-inch bowl industrial centrifuge, they were able to remove 64 to 84 percent of the influent algae (200 mg/l), depending on the throughput rate. Power requirements varied inversely with the original algal concentrations. They concluded that the use of centrifugation to separate algae was limited by the high initial cost of the units and by operational power requirements, although development of new power sources (atomic) and changes in centrifuge technology might make the use of centrifuges economically feasible in the future. Centrifuges are

desirable concentrating and dewatering devices because of their simplicity of operation and high product quality.

Drying

Algae samples for distribution normally were obtained by draining the contents of the sedimentation tank onto a 2-foot by 2-foot section of cloth supported on a wire frame. A 2-inch layer of 5 percent solids algae sludge dried to about 85 percent solids in two to three days. On one occasion an attempt was made to quantify the drying rates of the algal slurry on various types of surfaces. Three layers of algae, 0.5, 0.75, and 1.25 inches thick, were tested on 12-inch diameter circles of asphalt, cloth, black plastic, and sand. Table 19 shows the number of hours required for the sludge to go from about 6.4 percent solids to 80 percent solids. The sand drying method was by far the fastest, but it also resulted in a product having a considerable amount of entrapped sand particles. The asphalt surface would probably be the most practical because the algae could be applied and scraped off with less difficulty. The foregoing study was conducted in May 1970 and will be repeated to determine seasonal variations in drying time.

A dewatered sample of algae sent to the De Laval Corporation for spray drying contained 10 percent solids and was dried to 90 percent solids with no difficulty. Operators of the unit did not expect any problems when drying an algal sludge containing 20 percent solids.

Regrowth Studies

Laboratory studies designed to determine whether nitrogen removal actually lowered the potential of tile

TABLE 19

NUMBER OF HOURS REQUIRED TO REACH 80% SOLIDS, FROM ORIGINAL SAMPLE CONTAINING 6.4% SOLIDS

Slurry Depth (inches)	Type of Surface			
	Asphalt	Cloth	Black Plastic	Sand
0.5	46	36	42	24
0.75	No sample	43	No sample	50
1.25	72	65	73	80

drainage water to stimulate or support additional algal growth in waters of the Sacramento-San Joaquin Delta were integral parts of the nitrogen removal investigation. The objectives of these regrowth studies were to determine if: (1) tile drainage stimulated growth of algae in Delta waters; (2) if the treatment processes studied at Firebaugh lowered the stimulatory effect; and (3) if the waste did prove stimulatory, could the effect be attributed to any particular constituent (or constituents)? Some of the preliminary results of these studies were presented by Brown, et al, 1969, and a comprehensive final report will be published in 1971. A review of results pertinent to algal stripping will be discussed here.

Fourteen separate algal assays were conducted during a one-year period at EPA (Alameda) and DWR (Bryte) laboratories. The general procedure in these assays included collecting water samples from the Sacramento-San Joaquin Delta near Antioch (site of proposed drain discharge) and transporting the samples to the laboratory where they were mixed with various percentages (from 1 to 50 percent) of treated (algal growth and harvesting) and untreated tile drainage. Triplicate flasks of the different mixtures were then incubated at 24± 1°C and under continuous fluorescent lighting (approximately 300 to 400 ft-c). No additional algal seed was added. Growth responses of the indigenous algae were then followed by observing daily changes in in vivo chlorophyll fluorescence as measured directly in a fluorometer without chlorophyll extraction. The samples were incubated until the chlorophyll levels had peaked. The peak values of the mixtures of tile drainage and Delta water were compared with those of controls, which contained only Delta water. A statistical examination of the data indicated that in vivo fluorescence was directly correlated to cell number ($r = 0.69$) and that either peak fluorescence or change in fluorescence could be used to determine the extent of growth response in the cultures.

Figure 53 illustrates the results of a typical batch algal assay of an algal pond effluent with a relatively high inorganic nitrogen concentration of 3.7 mg/l. In this particular experiment, all additions of tile drainage stimulated algal growth in the Delta waters; although the peak fluorescence values for any particular dilution were lower with treated effluent than with untreated tile drainage. The results also show that the maximum algal growth occurred when 0.8 mg/l nitrogen was added. Above this level, some other nutrient (or light) presumably became limiting. Cell counts made at the beginning and end of the assay indicated that the cultures retained the original proportion of algal groups

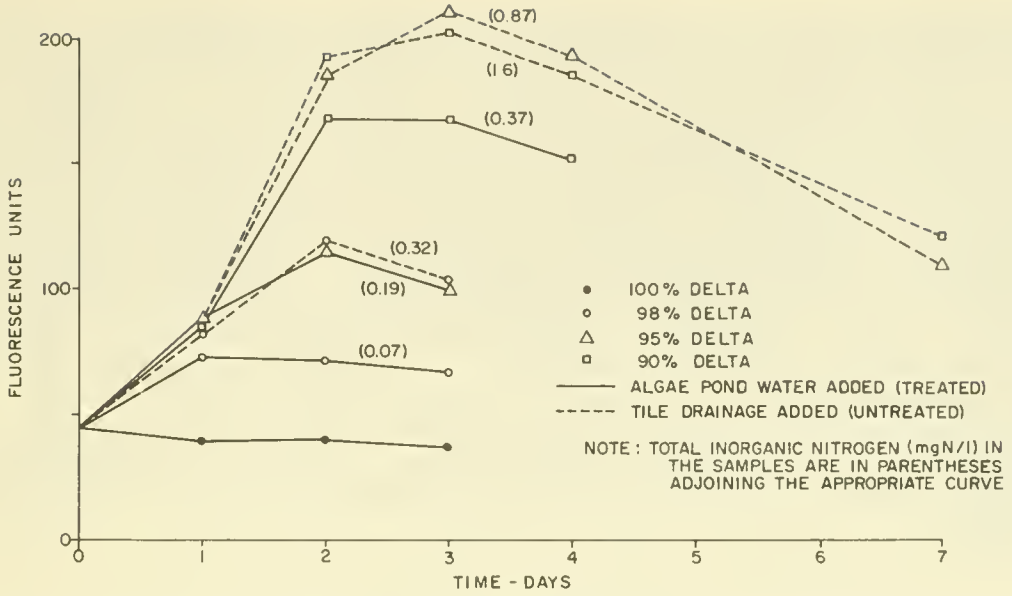


FIGURE 53-ALGAL GROWTH RESPONSES OF VARIOUS COMBINATIONS OF UNTREATED AGRICULTURAL TILE DRAINAGE AND "ALGAL STRIPPED" WATER MIXED WITH SAN JOAQUIN RIVER WATER (DELTA)

(75 percent diatoms, 25 green and blue-green) during the incubation period, regardless of the composition of the culture medium.

The results of all assays using algal system effluent showed that treatment by such a process reduced the biostimulatory nature of the tile drainage. When the dissolved inorganic nitrogen content of the effluent was reduced to 2 mg/l or less, additions of as much as 20 percent of algal system effluent usually had little stimulatory effect on algae in Delta waters. Peak fluorescence values of Antioch controls (incubated samples of Delta water only) were relatively stable throughout the 12-month period, although the initial levels varied seasonally. Samples collected in the winter and early spring contained 0.5 to 1.0 mg/l inorganic nitrogen and had low initial fluorescence levels; whereas, samples collected during the summer and fall had little inorganic nitrogen (0.2 mg/l), and the initial fluorescence level was similar to the final level. At the low inorganic nitrogen concentration found in water from the western Delta during the summer months addition of nitrogen-rich tile drainage caused stimulation of algal growth in laboratory cultures.

In many of the assays, the treated tile drainage was restored to its original nitrogen level by the addition

of analytical grade potassium nitrate. Comparison of fluorescence values in cultures grown in the "respiked" drainage with those grown in untreated tile effluent did not show any significant difference in stimulatory effect; the data indicated that nitrogen was the important biostimulatory substance in the drainage, at least with respect to the conditions of the test. In many of the assays, water treated by bacterial denitrification and algal stripping were compared for their effect on algal growth. Denitrification results in the removal of nitrogen only; whereas, theoretically, algal stripping leads to the removal of nitrogen as well as other nutrients essential for algal growth. Statistical analyses of the results demonstrated that, if the nitrogen levels of the effluent were comparable, growth responses in the cultures were similar. That is, effluent nitrogen level, not the method of treatment, was the important factor.

Botulism Studies

The results of botulism studies conducted by California Department of Fish and Game are contained in a progress report titled "Applied Botulism Research Including Management Recommendations", published by the Wildlife Management Branch in January 1970. The study was initiated to determine whether algal growth units would provide a suitable habitat for the bacterium, Clostridium botulinum, Type C. This organism, which often occurs in shallow bodies of water in the San Joaquin Valley, can cause waterfowl mortalities, where the necessary ecological conditions are present. The most significant such condition is complete anaerobiosis, although Clostridium botulinum can survive in the presence of oxygen if an electron acceptor or reducing substance is also present. The required anaerobic condition in waterfowl areas normally is satisfied by the remains of dead organisms, either plant or animal, that are killed as the water level in the area is raised and lowered. Carcasses of birds, fish, and mammals are the primary means whereby the botulism bacterium is perpetuated, because they provide anaerobic conditions necessary for its growth. The botulism-infected maggots living in the carcasses are ingested by healthy waterfowl, which then sicken and die. The botulism outbreak is then continued as the bacteria multiply in the carcasses of the recently killed waterfowl.

Fish and Game personnel made periodic trips to the Firebaugh site and sampled for the presence of Clostridium botulinum with consistently negative results. Injection of pure toxin into the pond water showed that the toxic effects of the material disappeared within a period of time varying from a few minutes to four hours.

Although Clostridium botulinum was never observed in the ponds at the IAWTC, even in the pond containing soil from a known botulism area, the report concluded that the shallow algae ponds could be potential problem areas if proper precautions were not exercised. It was recommended that the ponds be constructed with levee slopes of 30° or more and that carcasses of birds, fish, or mammals be removed immediately. If these recommendations are followed, waterfowl will be able to use the algae growth ponds as resting areas without suffering detrimental effects.

CHAPTER V - DISPOSAL

The use of photosynthetic organisms to remove nutrients from agricultural drainage water produces an algal by-product. Estimates for an algal stripping plant to treat the total tile drainage from the San Joaquin Valley in the year 2000 indicate that between 66,000 and 83,000 tons of algae will be produced annually, or about 200,000 cubic yards of material which would require disposal each year. This material may prove to be a valuable by-product and thus defray part of the total treatment cost. Actual project research into the disposal possibilities was limited to reviewing literature, making an economic evaluation of potential markets, and supplying product samples to interested companies. Thirty-five companies with a potential interest in an algal product were contacted to determine the extent of their interest. These included feed and grain dealers, representatives of the chemical industry, and companies distributing or producing soil supplements. About one-third indicated they would like samples for further evaluation. These samples were provided. The results of their studies have not been received as yet. In the following paragraphs, some of the possible uses will be discussed, along with estimated market potentials.

Animal Food Supplement

Marine algae have often been used to provide food for humans and livestock, especially in the Asiatic areas of the world. Recently, interest has been shown in the possible uses of unicellular algae as a protein source, especially for livestock feeding. This interest arises from the fact that algal production is a more efficient use of land than is conventional farming (in terms of biomass produced per unit area) and substrates for algal growth are often readily available in the form of domestic sewage and other liquid wastes. Usually these substrates are fairly complete and need only minimal supplemental growth factors. Although algae produced from these systems may have a variable composition, normally their protein content is quite similar to that of commonly used protein supplements, soybean meal and cottonseed meal, for instance. An idea of the composition of an algal product can be obtained from the results of analyses described in the following paragraphs.

Hintz, et al, (1966) reported on the analysis of several samples of sewage-grown mixtures of Scenedesmus and Chlorella. The results of these analyses are shown in Table 20. The composition is similar to that reported by other authors, although the composition varies with the

TABLE 20

COMPOSITION OF ALGAE GROWN ON SEWAGE[a, b]

Component	Number of Samples	Percent
Crude Protein	25	50.93 ± 0.68[c]
Crude Fiber	25	6.20 ± 0.41
Ether Extract	25	6.01 ± 0.40
Ash	25	6.24 ± 0.74
Cellulose	10	3.33 ± 0.25
Lignin	10	4.21 ± 0.30
Calcium	10	1.93 ± 0.19
Phosphorus	10	2.22 ± 0.10
Silica	10	1.73 ± 0.21
Magnesium	Composite	1.60
Potassium	Composite	0.92
Iron	Composite	0.23
Sodium	Composite	0.23
Zinc	Composite	0.18
Aluminum	Composite	0.12
Manganese	Composite	0.03
Copper	Composite	0.01
Lead	Composite	0.01
Molybdenum	Composite	Not detected
Carotene	3	221.4 ± 59.2[d]

a - Hintz, et al, 1966
b - Percent dry matter
c - Standard error
d - µgm/gm

environmental conditions under which the algae are grown. The carbohydrate (fiber) portion of the cell is fairly stable; however, the fatty acid (ether extract) or lipid components are particularly variable. If algae are grown in a low nitrate medium, the cell develops fatty acid storage products at the expense of protein production. Milner (1948) reported that the protein fractions of Chlorella pyrenoidosa varied from 7.9 to 46.4 and the lipid fraction varied from 20.2 to 75.5 percent of the total cell material, depending on environmental conditions. The production of fats might lower the value of algae as a food supplement, but this should not occur in the algae stripping process where nitrogen is seldom a limiting factor.

Foree and McCarty (1968) summarized the results of various investigators reporting on the composition of freshwater algae. Table 21 illustrates the concentration of the various algal components. The percentage of ash was highest in the two species of Scenedesmus analyzed and was about 15 percent of the dry weight. Scenedesmus also had a higher proportion of protein, about 50 percent of the dry weight, than the other algae cultures listed.

During the feasibility studies at Firebaugh, relatively little analytical work was performed on the composition of the algae. Organic nitrogen concentrations ranged from about 2 to 12 percent of the volatile solids of algae produced in outdoor growth units, although it was normally in the range of 7 to 9 percent. Total phosphorus was usually 1 to 3 percent of the volatile solids. The difference between the algal product collected at Firebaugh and the algae shown in Table 21 was the high percentage of ash, usually about 30 to 50 percent of the total suspended material in the Firebaugh product. Analysis of the ash showed the existence of significant concentrations of carbonates, iron, phosphorus, chloride, sulfate, and magnesium. The primary anionic constituent was carbonate. The ash was present when ponds were operated at pH values near neutrality but was lowered to about 10 percent nonvolatile material when in-pond sedimentation was included in the pond design.

Amino acid profiles were run on two samples of oven-dried algae by means of automated column chromatographic analyses. The results are given in Table 22. Both samples contained all ten of the amino acids essential for animal growth. The values as determined by analyses are in reasonably close agreement with those reported by Fisher and Burlew (1953) for pilot plant cultures of Chlorella pyrenoidosa.

Samples of the algal product and tile drainage water were analyzed for the presence of pesticide by gas chromatography. Neither the algae nor the water contained detectable amounts of organic phosphorus pesticide, but the tile drainage had about 10 parts per trillion (ppt) of dieldrin. The dried algae product had 75 parts per billion (ppb) of unidentified chlorinated hydrocarbons, probably the decomposition products of chlorinated hydrocarbon pesticides.

Several feeding experiments have been reported in which algae have been used in place of the normal protein supplement. Hintz, et al (1966), fed various percentages of sewage-grown algae (mixed culture of Scenedesmus and Chlorella) to swine, cattle and sheep. Results obtained in this study showed that to ensure consumption the algae had to be pelleted with other feeds and that ruminants could digest about

TABLE 21

PROTEIN, CARBOHYDRATE, AND LIPID CONTENTS OF SOME FRESHWATER ALGAE
(From Foree and McCarty, 1968)

Algal Species	(% Dry Wt.) Ash	Number of Cultures	(% Ash-Free Dry Weight) Protein	Carbohydrate	Lipid	Reference
Chlorophyta						
Chlorella pyrenoidosa	5.60	13	39.6	19.3	41.1	Spoehr & Milner (1949)
Chlorella pyrenoidosa		17	37.4	34.0	28.6	Ketchum & Redfield (1949)
*Chlorella pyrenoidosa		8	16.1	35.0	48.9	Aach (1952)
*Chlorella vulgaris		5	19.2	53.6	27.2	Fogg & Collyer (1953)
Chlorella vulgaris	12.40	1	48	32	20	Ketchum & Redfield (1949)
Scenedesmus obliquus	15.38	2	51	23	26	Ketchum & Redfield (1949)
Scenedesmus basilensis	14.34	1	53	20	27	Ketchum & Redfield (1949)
Stichococcus bacillaris	9.61	6	41	38	21	Ketchum & Redfield (1949)
Stichococcus bacillaris	8.87	2	42.5	32.1	25.4	Milner (1953)
Chlamydomonas sp.	4.74	1	36.3	58.2	5.5	Milner (1953)

*Carbohydrate fraction calculated assuming 5% ash and protein + carbohydrate + fat = 100% ash-free dry weight.

Values presented are mean values for the number of cultures indicated.

TABLE 22

AMINO ACID COMPOSITION OF OVEN-DRIED RGP PRODUCT ALGAE

Amino Acid	Sample 1 - 10/15/69		Sample 2- 1/5/70	
	Percent of Total Amino Acid Conc.	mg N/mg Algae x 10^{-4}	Percent of Total Amino Acid Conc.	mg N/mg Algae x 10^{-4}
Lysine	7.6	3.2	5.7	1.1
Histidine[1]	2.2	1.3	1.9	0.5
Arginine[1]	7.8	5.5	5.1	1.6
Tryptophan[1]	1.1	0.3	1.0	0.1
Aspartic Acid	10.5	2.4	11.9	1.3
Threonine[1]	4.9	1.3	5.4	0.6
Serine	4.7	1.4	6.0	0.8
Glutamic Acid	10.5	2.2	13.1	1.2
Proline	5.7	1.5	5.2	0.7
Glycine	6.6	2.7	7.1	1.3
Alanine	9.5	3.2	8.4	1.3
Valine[1]	5.8	1.5	6.1	0.7
Methionine[1]	1.8	0.5	1.8	0.2
Isoleucine[1]	3.7	0.9	4.2	0.4
Leucine[1]	8.8	2.1	8.4	0.8
Tyrosine[1]	3.5	0.6	3.4	0.2
Phenylalanine[1]	4.8	1.0	5.6	0.5
Total	99.5	31.6	100.3	13.3

[1] Essential amino acid.

20 percent more of the crude protein than could swine. The study concluded that, although algae were not a high energy food, its high protein content and available minerals made it a potentially useful food supplement. Erchul and Isenberg (1968) evaluated the protein quality of various algal biomasses produced at a water reclamation experimental pilot plant. The products were mainly a mixture of Chlorella and Scenedesmus fed to rats to compare protein efficiency ratios (PER) to casein, soybean, and fish meal supplements. The algal biomass with the highest PER compared favorably with soybean meal. The variation in PER was apparently due to differences in digestibility.

Leveille, et al (1962), reported on the use of various algae (Chlorella, a mixture of Chlorella and Scenedesmus and Spongiococcum) as the sole protein source in rat and chick feeding studies. All the algae were inferior to soybean meal as a source of protein. The data showed them to be deficient in methionine for rats and chicks and the mixture to be deficient in glycine for chicks. Grau and Klein

(unpublished progress report, 1956) fed sewage-grown algae (Scenedesmus-Chlorella mixture) to chicks and found that growth was generally slower when algae was used in place of soybean meal. The use of alum-flocculated algae appeared to be especially detrimental to chick growth when the concentration of aluminum in the feedstuff exceeded 0.5 percent of the chickens' total food intake.

Researchers from the North American Aviation Company (1967) reported on the feeding of mixtures of Scenedesmus, Closteridium, and Chlorella (grown on sewage) to chickens as a substitute for soybean meal. Their results indicated that the protein value and feeding efficiencies of algae were comparable to soybean meal. The algae may actually be preferred by the chicken rancher because the algal pigment, xanthophyll, gives a deeper golden color to the skin, meat, and egg yolks. The algae grown at Firebaugh may be more suitable for poultry production because the salts included with the algae might provide some essential nutrients to the fowl. (Most poultry diets contain a small percentage of certain salts.)

Information in the preceding paragraphs indicates that algal meal may find a market as a substitute for such protein supplements as soybean, cottonseed, or fish meal, especially in poultry production, although more data are needed. The next step was to determine whether the potential market could absorb the production of the proposed algal stripping plant. In Table 23 the estimated maximum and minimum algal production is given for each five-year interval from 1975 to 2000. The data are based on projected drainage flows from the entire San Joaquin Valley and estimates of algal production per unit flow. The basic assumption is that the total drainage from the Valley will eventually require treatment. The data indicate that peak annual production may be between 66×10^3 and 83×10^3 tons of dry algae. According to the California Department of Agriculture (1965), the California poultry industry used more than 560×10^3 tons of protein supplement in 1963, while the total used by all feeding operations was almost one million tons. The prices paid for these animal feed supplements vary from $80/ton (cottonseed meal) and $100/ton (soybean meal) to $110/ton (meat and bone meal). If, as the tests indicate, algae can readily be substituted for soybean meal, the value of the by-product will be as shown in Table 23.

Use of Algae as a Soil Conditioner

Another market which can absorb the production of a full-scale algal stripping plant is the retail market for soil supplements to lawns, golf greens, etc. Its high

TABLE 23

ESTIMATED ALGAL PRODUCTION BY AN ALGAL STRIPPING PLANT, 1975-2000

Year	Thousands of Tons of Algae per Year		Approximate Value as Substitute for Soybean Meal	
	Maximum	Minimum	Maximum	Minimum
1975	13,300	8,410	$1,330,000	$ 841,000
1980	27,200	18,000	2,720,000	1,800,000
1985	44,300	29,610	4,430,000	2,961,000
1990	62,000	42,400	6,200,000	4,240,000
1995	75,300	54,100	7,530,000	5,410,000
2000	82,610	65,510	8,261,000	6,551,000

nitrogen content and various salts in the product, combined with its slow rate of decomposition, make algae a desirable lawn conditioner. The current wholesale price of a soil conditioner manufactured in San Jose, California, is about $100/ton. This figure is the cost of 50-pound sacks of the product intended to retail at $140/ton, 1970 prices, in Fresno, California. Railroad freight agents were hesitant about quoting shipping costs; however, the maximum prices would be as shown in Table 24 (shipped in 100-pound sacks). The exact shipping costs, which will be determined when algal product is given a freight classification, will have to be deducted from the value of the algae.

Sewage plant operators have tried to develop markets for their dried activated sludge, which is mainly composed of nonbiodegradable polysaccharide, protein, fat, and inorganic complexes (Hurvitz, 1957). According to a report by Foster D. Snell, Inc. (1969), the market for this product has decreased because such material is not competitive with other nitrogen sources, and many plants are unable to recover freight costs, let alone process costs. An algal product with higher nitrogen content (6 to 8 percent) than activated sewage sludge (1 to 2 percent) may find a more receptive market.

TABLE 24

RAIL FREIGHT RATES FOR 100 LB. ALGAE PACKAGES
FROM SAN FRANCISCO, CALIFORNIA

Destination	Amount Per 100 Lbs.	Amount Per Ton
Los Angeles	$1.30	$26.00
Portland	1.76	35.20
New York	4.31	86.20
Kansas City	3.23	64.60
Fresno	.82	16.40
Tacoma, Washington	1.98	39.60
Atlanta, Georgia	3.94	68.80

Miscellaneous Possible Markets for an Algal Product

Some other possible markets exist for algae, but current technological difficulties preclude their consideration as important market outlets. A brief outline of several of these uses follows:

1. Some interest has been expressed in feeding algae to organisms reared commercially in aquacultures. These organisms include freshwater fish and marine bivalves (clams and oysters). Development of these aquatic farms is now mostly in the talking and planning stages and could develop into a substantial market for algae.

2. Borgmann and Feeney (1948) isolated a sterol from Scenedesmus obliquus which had characteristics identical to those of chondrillasterol, a sterol isolated from a sponge. This algal sterol is one of the few naturally occurring compounds having the necessary configuration to serve as a starting point in the manufacture of cortisone, an important drug. More research will be required to determine whether this sterol is present in commercially valuable quantities in Scenedesmus quadricauda used in this project.

3. Use of algal protein in the adhesive industry has been suggested as a market for the product. Samples of Chlorella sent by Fisher and Little (1953) to a private laboratory to investigate

the possible uses of the protein fraction in algae showed that the protein was difficult to separate and that the molecular weight was too low to serve as a substitute for casein. Most chemical adhesive manufacturers are using synthetic compounds for adhesive production and show little interest in natural organic sources.

Use of Algae to Produce Methane Gas

Suggestions have been made that an algal product can be converted to usable energy through methane fermentation, with subsequent use of the methane gas to provide heat and/or electric power. In a comprehensive paper on this process, Oswald and Golueke (1960) concluded that this could be a practical means of converting solar energy to thermal or electrical energy. The problem with this type of system in tile drainage is the high concentration of sulfate present. This amounts to about 3,000 mg/l in the Alamitos tile system. Foree and McCarty (1968) studied the anaerobic decomposition of algae and concluded that, as long as dissolved sulfate is available, organic matter decomposes through the reduction of the available sulfate ion. Further stabilization of the material then occurs by methane fermentation, as long as the sulfide concentration is not toxic to the bacteria. Little or no methane was produced in the decomposition of algal cultures containing about 2,000 mg/l dissolved sulfate. The presence of this inhibitory action may preclude the use of this method of algal disposal in the projected agricultural wastewater treatment plant, but an investigation would be required to ascertain the feasibility, methodology and problems associated with such a system.

CHAPTER VI - PROCESS EVALUATION

The studies at the IAWTC have shown that the algal stripping process can be a reliable means of reducing the nitrogen concentration in agricultural tile drainage. Because the algal process involves more than one step, and because each step may have alternatives, this section of the report will assign tentative estimates of nitrogen removal at each step, as well as total nitrogen removal by the system. Also, the section will include some preliminary design criteria and estimates of treatment costs based on facilities built and operated according to these criteria.

Removal Efficiencies

As shown in Figure 29, the algal cultures assimilated an average of 80 percent of the total influent nitrogen (80 percent of 20 mg/l, or about 16 mg/l) from July through December 1969. This period, which covered the temperature range noted at the site, was selected because of standardized conditions in operation of the growth units. This 80 percent average is somewhat misleading because it includes data from the start-up period of the run -- algae growing at steady-state conditions assimilated about 90 percent, or 18 mg/l, of the influent nitrogen with an effluent of 2 mg/l, or less, total nitrogen. The amount of nitrogen assimilated by the algae increased steadily as the study progressed, probably because of more complete understanding of the system and natural selection of a strain of algae more adapted to the waste and to the environment of the San Joaquin Valley. The average amounts of influent nitrogen assimilated by the (best) pond during steady-state conditions for all minipond runs are shown in Figure 54. During 1969 from 16 to 21 mg/l of nitrogen were assimilated, as compared to 7 to 10 mg/l in the earlier runs. Preliminary (1970) operational data obtained at the time this report was being prepared indicate that the algal system can remove 27 to 28 mg/l from a 30 mg/l nitrogen influent.

Based on data from the feasibility phase of the project, minimum nitrogen assimilation (20 mgN/l influent) will be 16 mg/l and maximum assimilation about 18 mg/l. If 95 percent of the algae can be separated from the liquid phase (a level consistently achieved by the flocculation-sedimentation unit tested at the IAWTC), then the total nitrogen remaining in the effluent would be on the order of:

$N_T = N_D + N_A$ where N_T = total effluent N; N_D = dissolved effluent N; and N_A = nitrogen in effluent algae or (% algae remaining)(original algal conc.)(% N in algae)

Maximum N_T = 4 mg/l + (0.05 remaining)(250 mg/l)(0.08 N in algae)

= 5 mg/l N,

Minimum = 2 mg/l + (0.05 remaining)(250 mg/l)(0.08 N in algae)

= 3 mg/l N

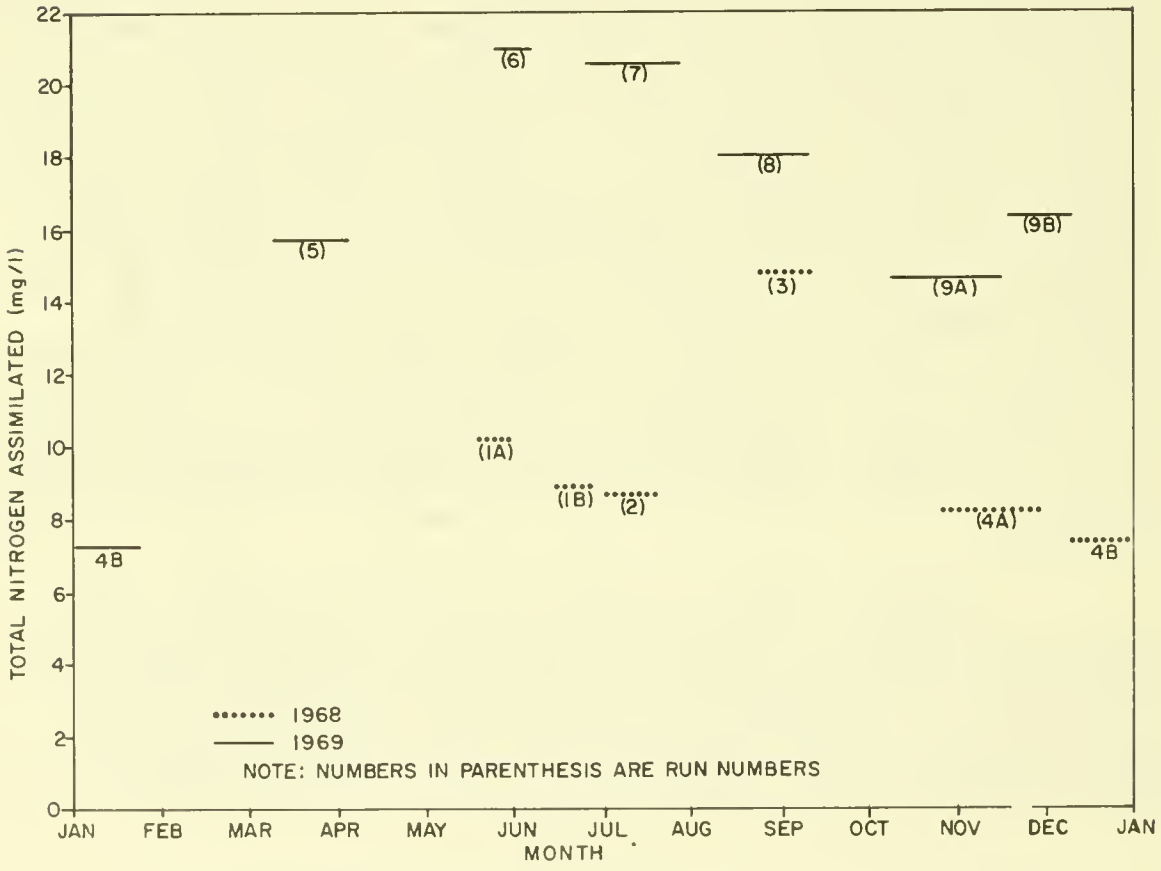


FIGURE 54 - NITROGEN ASSIMILATION IN VARIOUS MINIPOND RUNS

These figures were based on 20 mg/l total N in the influent. Thus, overall nitrogen removal efficiencies will range from 75 to 85 percent. Depending on water temperatures, growth unit detention times of 5 to 16 days will be required to achieve these removal rates.

Process Configuration

From the data contained in this report, a tentative process configuration can be drawn. The configuration is preliminary in that data from the operational phase of the study were not considered. Figure 55 illustrates the flow diagram of an algal stripping plant. Shallow growth units are followed by a sedimentation process which removes 95 percent of the suspended biomass. The effluent from the sedimentation tanks leaves the plant site and the algal slurry goes to a vacuum filter for dewatering to about 20 percent solids. The effluent from the dewatering device is recycled to the head of the sedimentation area while the sludge is dried to 85 to 90 percent solids by air or flash driers. The dried product is then removed from the site to be sold as a marketable by-product.

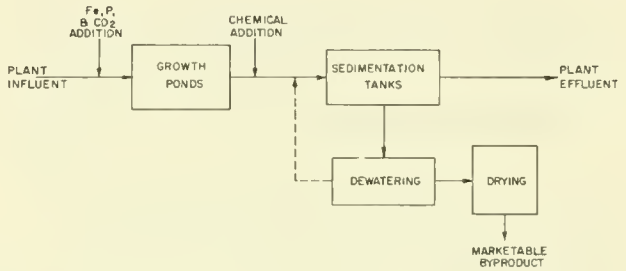


FIGURE 55-FLOW DIAGRAM OF ALGAL STRIPPING PLANT

Figure 56 illustrates a schematic design of an algal growth unit. The flow enters and leaves the unit by gravity through concrete pipes. Iron, phosphorus, and CO_2 are added to individual growth units at the mixing pumps. Outdoor propeller-type pumps perform four hours mixing per day. To reduce the required number of mixing pumps, it is proposed that the ponds be constructed in units of 12 with a central mixing station capable of mixing two ponds at a time for four hours. This type of design is shown in Figure 57. A series of automated valves would rotate mixing to the desired ponds. An in-line sedimentation area included in the design of the growth unit will remove much of the heavier suspended solids.

Cost Estimates

The feasibility studies conducted at the IAWTC were not designed to provide definitive cost data for an algal stripping system; however, some preliminary process cost estimates can be made. These estimates will undoubtedly be revised as more data become available from 1970 operational studies.

This is especially true in the case of pond mixing. Results demonstrated that inorganic carbon addition may be more important than mixing.

Predicted seasonal variation in flows and nitrogen concentrations of tile drainage from the San Joaquin Valley (see Figure 58) and climatic factors such as temperature and sunlight indicated that April was the critical month for sizing the growth units. During this month, flows will be high, nitrogen concentration should be about 30 mg/l, and sunlight and temperature conditions will not be optimum.

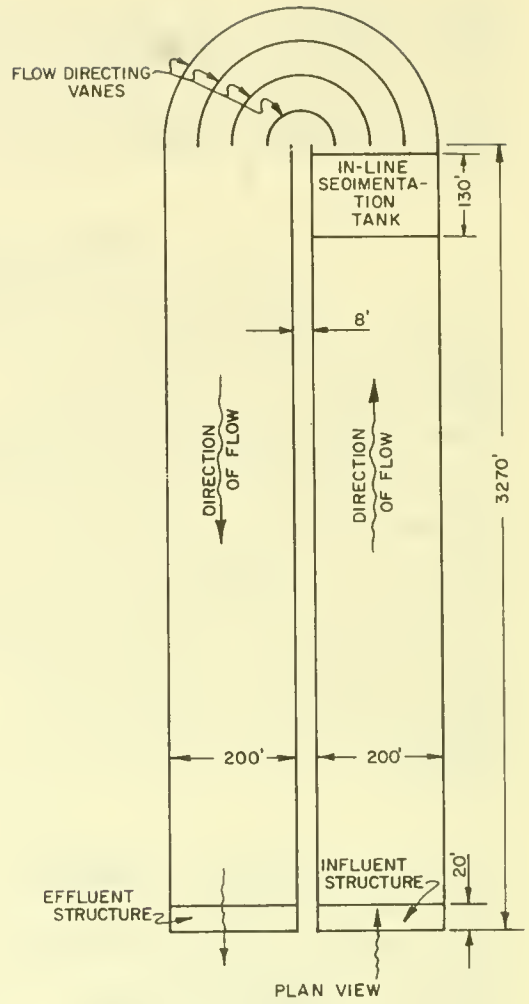


FIGURE 56-ALGAE GROWTH POND

Several factors were used in obtaining these estimates:

1. All costs were based on January 1970 dollars.

2. Debt service was calculated for 50 years at 5 percent.

3. Costs per million gallons treated were calculated by dividing the total annual cost (debt service plus operations and maintenance costs) for a full-capacity plant by the estimated annual flow of the San Luis Drain.

4. An engineering and contingency factor of 57 percent was assumed for capital cost items whose design and operation was based on experimental data obtained at the treatment center. A lower engineering and contingency factor was used for items common to waste treatment facilities and for those items which can be purchased from the manufacturer. These engineering and contingency factors are shown in Table 27.

5. The land required for a full-capacity plant is to be purchased at the beginning of the project at $500 per acre.

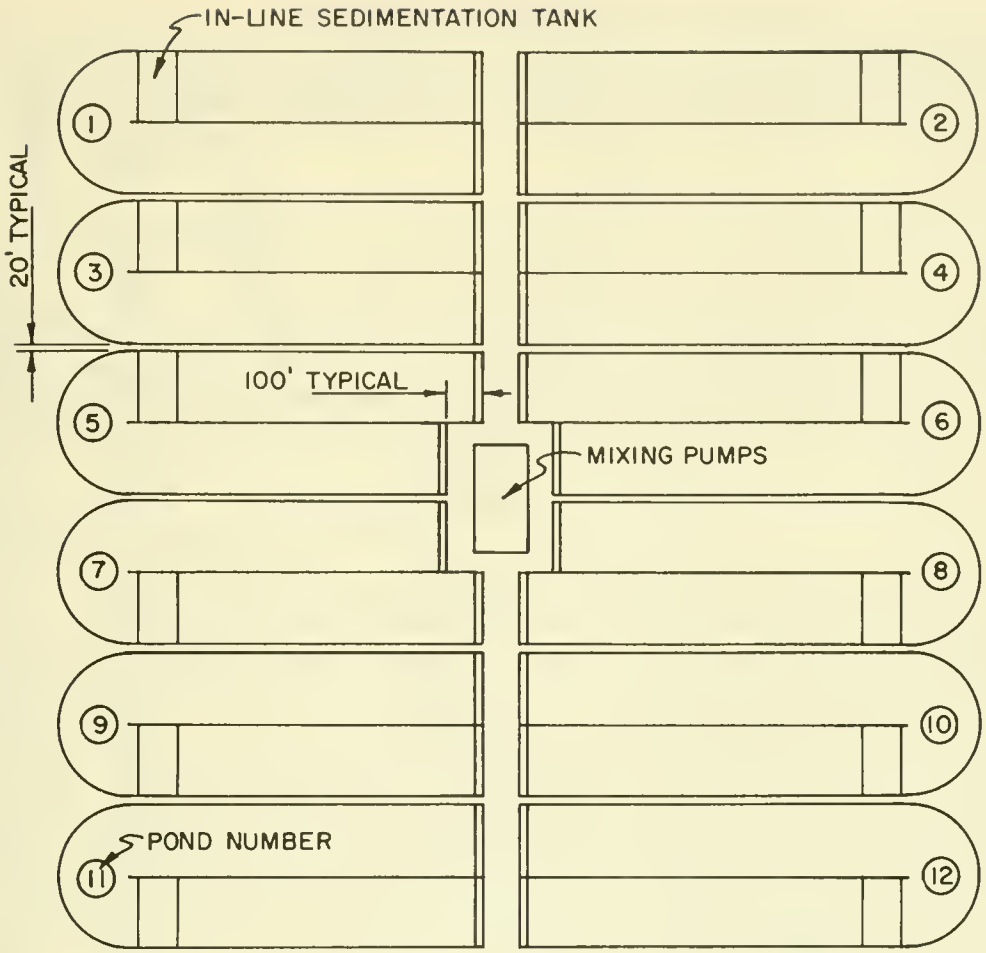


FIGURE 57 - ONE OF THREE 12-POND GROUPS CONSTRUCTED PER PHASE

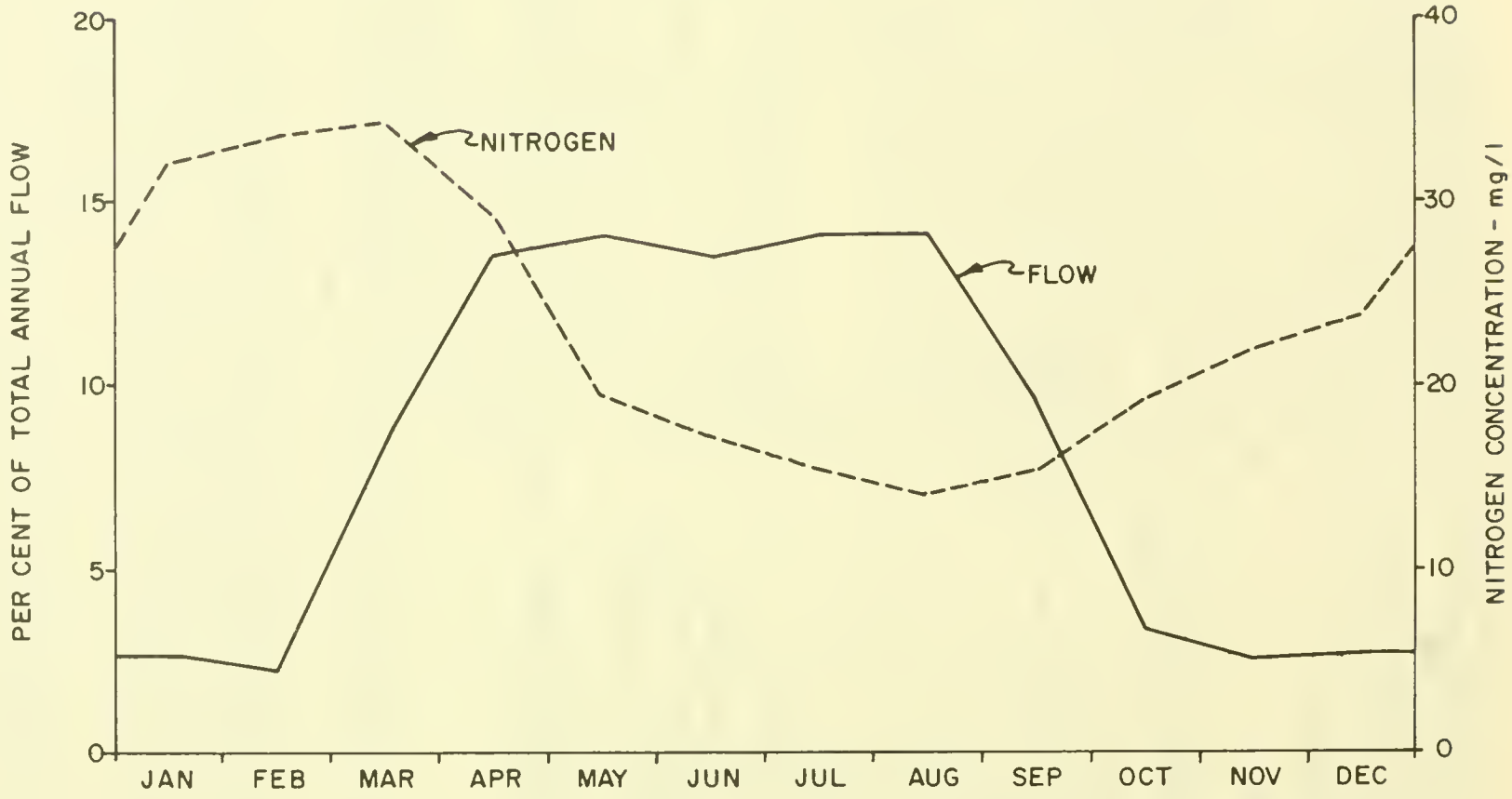


FIGURE 58-PREDICTED SEASONAL VARIATION OF TILE DRAINAGE FLOW & NITROGEN CONCENTRATIONS FROM SAN JOAQUIN VALLEY, CALIFORNIA

6. The laboratory and office buildings and maintenance and storage areas are basically the same as those at the San Jose, California, Sewage Treatment Plant. Capital costs for these facilities are based on information published by Guthrie (1969).

7. Electric power costs were calculated on the basis of $.01/kw-hr.

8. General plant operation and maintenance (O&M) costs were based on curves published by Smith (1967) for trickling filter O&M. It was assumed that the general plant O&M costs included replacement of power costs for all plant operations except for the items indicated in Table 26.

9. The cost of reaeration was taken from figures published by Smith (1967).

The individual ponds are designed according to the criteria presented in Table 25, with a mean channel length of approximately 6,500 feet. The influent and effluent structures, the in-line sedimentation tank, and flow-directing vanes are all made of concrete. In addition, wherever a concrete structure and the growth pond proper meet, a 20-foot concrete apron is built. Although an in-line sedimentation tank was not built into the rapid growth pond operated at the IAWTC, evaluation of a sedimentation unit used in conjunction with the growth pond indicated that a device of this type would be beneficial to algal growth. The in-line sedimentation system included in this proposed pond design is routinely used in operations requiring sedimentation facilities but may not be the best solution of the problem of removing excess suspended material from the growth unit. With the exception

TABLE 25

ALGAE STRIPPING DESIGN CRITERIA

April Growth Pond Hydraulic Detention Time	8 days
Growth Pond Depth	1 foot
Growth Pond Width	200 feet
Mixing Velocity	1 fps
Duration of Mixing	4 hrs/day
In-line Sedimentation Tank	
Surface Loading	5,000 gpd/ft^2
Detention Time	15 minutes
Growth Chemical	
Fe	2 mg/l
P	2 mg/l
CO_2	800 lbs/million gal.

of the structures and aprons, the pond bottoms are assumed to be 22 percent soil cement and 78 percent compacted native soil by volume. The ratio is intended to prevent seepage into or out of the ponds. The levee which divides the pond is 2 feet high and has a crest width of 8 feet wide and 1.5-to-1 side slopes. It is constructed from the soil excavated from the influent and effluent structures and the in-line sedimentation tank. The flow-directing vanes are 2 feet high and 8 inches thick and built on 50-, 100-, and 150-foot radii. Ponds are separated from each other on the long sides by a 3-foot high levee with a 20-foot crest and 1.5-to-1 side slopes.

The surface loading on the flocculation-sedimentation tank is considered to be 900 gpd/ft^2, with 10 mg/l ferric sulfate and 0.5 mg/l cationic polyelectrolytes (Calgon - Cat-Floc) as the chemical additions. Loading on the vacuum filter is 0.2 gpm/ft^2, with capital and O&M cost estimates supplied by the Eimco Corporation. One-half of the sludge from the vacuum filter goes to 7,000 pounds H_2O/hr driers, and the remaining half is air-dried. Cost estimates for the driers were provided by the De Laval Separator Company.

Table 26 lists the capital costs of the various components of the algae stripping system and Table 27 illustrates the total costs of these components for treating a million gallons of tile drainage. The estimated net cost of treatment is $135 per million gallons, which includes the recovery of some money through the sale of an algal product. The product recovery figure was based on producing 1400 pounds of algae per million gallons of water and selling the product at $60 per ton.

The costs shown in Tables 26 and 27 were obtained by assuming that the plant would be built to treat the estimated maximum annual quantity of about 50,000 million gallons for the San Luis Drain. Because the estimated ultimate annual flow of the facility will not be attained until about 30 years from the time the initial drainage reaches the treatment plant, another approach would be to construct the plant in stages paralleling the buildup of drainage flows. For a treatment plant constructed in phases of equal size (each additional phase to be constructed during the year when drain flow reaches 90 percent of existing plant capacity), the average net cost for treatment over the first 50 years of operation would probably be higher than the estimate shown above. This increased price is because part of the plant would be idle at all times.

These preliminary cost estimates were obtained to determine which aspects of the process were the most costly. With this information, the operational studies of 1970 were

TABLE 26

TREATMENT COSTS FOR ALGAE STRIPPING

Item	Dollars Per Million Gallons	Percent of Total Cost
Capital		
Production		
Growth Pond	55	30
In-line Sedimentation Tanks	5	3
Chemical Feed Pumps	1	1
Mixing System	39	22
Harvesting		
Separator	1	1
Dewatering	1	1
Drying	6	3
Other		
Land, Building, Miscellaneous	6	3
Annual O&M Costs		
General	21	12
Drying, Dewatering & Growth Chemicals	27	15
Mixing Power	16	9
TOTAL	$177/mg	100
Minus By-product Income	-42	
Net Cost of Treatment	$135/Million gallons	

directed toward reducing the cost of the more expensive items (mixing, land preparation, etc.), while maintaining the maximum level of nitrogen removal.

The cost estimate presented in this section was based on high-rate algal growth units. The other algal system studied at the site -- the "symbiotic", soil-lined ponds -- has not had a cost estimate, primarily because of the unknown nature of the systems involved. Data obtained during the 1970 operational studies will provide some information on the questions of reliability and the mechanisms involved. With this data cost estimates can be prepared. Because the system is simple and because it requires no mixing and little chemical addition for growth, costs for this process will be considerably lower than for the high-rate algal growth system.

TABLE 27

CAPITAL COST FOR ALGAE STRIPPING

Number	Item	Capital Expenditure - 1970 Dollars
	Production	
1	Growth Ponds	$ 50,900,000
2	In-line Separation Tanks	5,000,000
3	Chemical Feed Pumps	125,000
4	Mixing System	37,500,000
	Harvesting	
5	Separator	250,000
6	Dewatering	1,250,000
7	Drying	6,125,000
	Other	
8	Land Acquisition	5,000,000
9	Buildings	750,000
10	Miscellaneous	157,000
	Total	$107,057,000

Notes: (1) Items Nos. 2,3,5,6,7 and 9 include a 25 percent engineering and contingency factor.

(2) Items Nos. 1, 4 and 10 include a 57 percent engineering and contingency factor furnished by the U. S. Bureau of Reclamation, in its "reconnaissance" estimate.

(3) No engineering and contingency factor was applied to land cost.

CHAPTER VII - AREAS FOR FUTURE INVESTIGATION

Many of the questions concerning technical feasibility have been answered by the study at Firebaugh and other answers will result from current operational studies. There are, however, areas of interest which cannot be fully investigated during the current project and which warrant further study. The proposed studies will be more meaningful when a combined drainage facility provides larger quantities of water. Among the areas of interest are:

1. A redesigned algal system which may reduce the land area and modify the type of growth units necessary for nitrogen removal.

The basic idea for this type of system was derived from personal communication with Dr. William J. Oswald, Professor of Environmental Health Sciences and Sanitary Engineering at the University of California, Berkeley. The type of unit suggested by Dr. Oswald would consist of a number of ponds in series, each containing different depths of water. The first pond would be relatively deep, with light as the factor limiting algal growth. This pond would only receive phosphorous addition and would not require mixing. The next pond would be shallower but light would still be the only limiting factor. The last pond in the series would be similar to the rapid growth pond used in the present study (shallow, with mixing, etc.), and nitrogen would be the constituent limiting growth. Nitrogen assimilation in the earlier ponds would permit the use of short detention times in the final unit. Theoretical considerations indicate that a system of this type could reduce the land area required in a conventional system as much as 50 percent.

2. Use of the drain as a treatment system has been described by Goldman, et al (1969), in which the drainage canals and holding reservoir are part of the process.

Because of nutrients contained in the water, the optical clarity of the drainage and the use of relatively shallow, lined canals, some natural algal growth (either planktonic or sessile), estimated at 15 to 60 mg/l, will occur to remove some nitrogen. If this growth is encouraged by nutrient additions and/or modification to the canal to increase turbulence, as much as 100 mg/l of algae could possibly be grown in the canal prior to Kesterson Reservoir (a holding reservoir). This could remove about 8 to 9 mg/l nitrogen. Then if Kesterson were redesigned to include both holding capability and improvements for increasing algal growth, it could conceivably remove the remaining nitrogen. A separation

plant would then be placed at the outlet of the reservoir. If these facilities are included in the drainage system, considerable savings in land costs may be realized.

3. Use of the algae growing capability of the drain and of Kesterson Reservoir with the anaerobic denitrification filter process as a final polishing step.

In the anaerobic system, an organic carbon source is added to the drainage and the water passed through a column containing some type of aggregate. Anaerobic bacteria attached to the media reduce the nitrate to nitrogen gas, which is evolved to the atmosphere. Studies at Firebaugh have demonstrated that algae entering the columns can increase dissolved organic and ammonia nitrogen, presumably by bacterial decomposition of algal protein. Since algae undoubtedly will grow in the drain, some provision for their removal will have to be incorporated in the design of an anaerobic system. If algal growth is encouraged, the dual system may reduce costs and provide a more reliable system for nitrogen removal. An unpublished communication from Dr. Oswald contains reference to the following possible benefits from the dual system:

a. Back-up coverage is provided in case of breakdown of one of the systems.

b. The algal system can remove ammonia nitrogen produced by decomposition of bacterial cells in the filter.

c. Recreational use may be provided by the ponds.

ACKNOWLEDGMENTS

The following list includes the personnel actively involved in the algae stripping project at the IAWTC.

Algae Stripping Project Under the Direction of

Louis A. Beck . . . . Engineer, California Department of Water Resources
Donald Swain . . . . . Engineer, U. S. Bureau of Reclamation
Percy St. Amant . . . . . Engineer, Environmental Protection Agency

Conducted by

Randall L. Brown . . Biologist, California Department of Water Resources
Bruce A. Butterfield . Engineer, California Department of Water Resources
Joel Goldman . . . . . Engineer, California Department of Water Resources
James F. Arthur . . . . . Biologist, Environmental Protection Agency
James R. Jones . . . . . Engineer, U. S. Bureau of Reclamation

Assisted by

Robert G. Seals . . . . . Chemist, Environmental Protection Agency
William R. Lewis . . . Chemist, California Department of Water Resources
Dennis L. Salisbury . . . . . Engineering Technician, California Department of Water Resources
William L. Baxter . . . Laborer, California Department of Water Resources
Clara P. Hatcher . . . . . Laboratory Aid, California Department of Water Resources
Elizabeth J. Boone . . . . . Laboratory Aid, California Department of Water Resources
Linda S. Harrington . . . . . Laboratory Aid, California Department of Water Resources

Consultants to the Project

Dr. William J. Oswald . . . . . University of California, Berkeley
Dr. Clarence G. Golueke . . . . . University of California, Berkeley
Dr. Perry L. McCarty . . . . . Stanford University, Palo Alto

Report Prepared by

Randall L. Brown . . . . . California Department of Water Resources

LIST OF REFERENCES

1. Aach, H. G. 1952. Waschstrum und Zusammensetzung von Chlorella pyrenoidosa. Archiv fur Mikrobiologie, 17:213-246.

2. American Public Health Association. 1965. Standard Methods for the Examination of Water and Waste Water, 12th edition.

3. Bergmann, W. and R. J. Feeney. 1950. Sterols of Algae. I - the occurrence of chondrillasterol in Scenedesmus obliquus. Jour. Org. Chem. 15:812-814.

4. Black, A. P., F. B. Birkner, and J. J. Morgan. 1965. Destabilization of dilute clay suspensions with labeled polymers. Journal American Water Works Assoc. 57:1547.

5. Bogan, R. H., O. E. Albertson, and J. C. Pluntz. 1960. Use of algae in removing phosphorus from sewage. Pro. Am. Soc. Civil Engrs., J. Sant. Engr. Div., SA5, 86:1-20.

6. Bohna Engineering and Research, Inc. 1970. Unpublished report entitled - Filtration of algal suspension. Submitted to California Department of Water Resources.

7. Brown, R. L., R. C. Bain, and M. G. Tunzi. 1969. The effects of nitrogen removal on the algal growth potential of San Joaquin Valley agricultural tile drainage effluents. Presented at the Fall Meeting of the American Geophysical Union, December 15-18, 1969.

8. California Crop and Livestock Reporting Service. 1965. Poultry and Hatchery Production in California, Summary for 1963.

9. California Department of Agriculture. 1970. Applied Botulism Research, including Management Recommendations Progress Report. 87 pages.

10. Chemical Rubber Publishing Company. 1951. Handbook of Chemistry and Physics. 32nd edition.

11. Cramer, M. and J. Meyers. 1948. Nitrate reduction and assimilation in Chlorella pyrenoidosa. J. Gen. Physiol. 32:93-102.

12. Erchul, B. A. F., and D. L. Isenberg. 1968. Protein quality of various algal biomasses produced by a water reclamation plant. J. Nutrition. 95:374-380.

13. Fair, G. M., J. C. Geyer, and D. A. Okum. 1968. Water and Wastewater Engineering, Volume 2. Water Purification and Wastewater Treatment and Disposal. John Wiley and Sons, Inc. New York.

14. Fisher, A. W. and J. S. Burlew. 1953. Nutritional Value of Microscopic Algae. In Algal Culture from Laboratory to Pilot Plant. J. S. Burlew, (ed.). Publ. 600, Carnegie Inst. of Wash. pp. 303-310.

15. Fisher, A. W. and A. D. Little. 1953. Algae as industrial raw materials. In Algal Culture from Laboratory to Pilot Plant. J. S. Burlew, (ed.). Publ. 600, Carnegie Inst. of Wash. pp. 311-315.

16. Fitzgerald, G. P. 1961. Stripping effluents of nutrients by biological means. Transactions of the 1960 Seminar on Algae and Metropolitan Wastes. Robert A. Taft, San. Engr. Ctr. Cincinnati. pp. 136-139.

17. Fogg, G. E. 1947. Nitrogen Fixation by Bluegreen Algae Endeavor. 6:172-175.

18. Fogg, G. E. And D. M. Collyer. 1953. The accumulation of lipides by algae. In Algal Cultures from Laboratory to Pilot Plant. J. S. Burlew, (ed.). Publ. 600, Carnegie Inst. of Wash. pp. 177-181.

19. Fogg, G. E. and M. Wolfe. 1954. The nitrogen metabolism of the blue-green algae (Myxophyceae). Symposium Soc. Gen. Microbiol. 4:99-125.

20. Foree, E. G. and P. L. McCarty. 1968. The decomposition of algae in anaerobic waters. Technical Report No. 95, Stanford University, Dept. of Civil Engr.

21. Foster D. Snell, Inc. 1969. Feasibility of hydrolysis of sludge using low pressure steam with SO_2 as a hydrolytic adjunct and utilization of resulting hydrolysate. Report prepared for the Fed. Water Qual. Admin.

22. Gest, H. and M. D. Kamen. 1948. Studies on the phosphorus metabolism of green algae and purple bacteria in relation to photosynthesis. J. Biol. Chem. 176:299-318.

23. Goldman, J. C., J. F. Arthur, W. J. Oswald, and L. A. Beck. 1969. Combined nutrient removal and transport system for tile drainage from the San Joaquin Valley. A paper presented before the National Fall Meeting of the American Geophysical Union, December 15-18, 1969. San Francisco.

24. Golueke, C. G. and H. B. Gotaas. 1958. Recovery of algae from waste stabilization ponds - II. U. of California (Berkeley) IER Senes 44 - Issue 8.

25. Golueke, C. G., W. J. Oswald, and H. K. Gee. Harvesting and Processing Sewage-Grown Planktonic Algae. 1964. San. Eng. Res. Lab., U. of California, Berkeley. SERL Report No. 64-8.

26. Grau, C. R. and N. W. Klein. 1956. Use of algae as a poultry feedstuff. Unpublished Progress Report for Fiscal Year 1955-56. Submitted to U. of California, Berkeley.

27. Guthrie, K. M. 1969. Capital Cost estimating. Chem. Engr., 76:6.

28. Hansen, S. P., G. L. Culp and J. R. Stukenberg. 1969. Practical application of idealized sedimentation theory in wastewater treatment. J. Water Poll. Contr. Fed., Vol. 41, No. 8, Part 1: 1421-1444.

29. Hemens, J. and M. H. Mason. 1968. Sewage nutrient removal by shallow algal stream. Water Research. 2:277-287.

30. Hintz, H. F., H. Heitman, Jr., W. C. Weir, D. T. Torell, and J. H. Meyer. 1966. Nutritive value of algae grown on sewage. J. Animal Science Vol. 25. 3:675-681.

31. Hurvitz, E. 1957. The use of activated sludge as an adjuvant to animal feeds. Engr. Bull., Ext. Series No. 94:395-414.

32. Ives, K. J. 1956. Electrokinetic phenomena of planktonic algae. Proc. Soc. Water Treat., Exam. 5:41.

33. Jewell, W. J. and P. L. McCarty. 1968. Aerobic decomposition of algae and nutrient regeneration. Technical Report No. 91. Stanford Univ. Dept. of Civl Engr.

34. Ketchum, B. H. 1939a. The absorption of phosphate and nitrate by illuminated cultures of Nitzschia closterium. Am. J. Botany. 26:399-407.

35. Ketchum, B. H. 1939b. The development and restoration of deficiencies in the phosphorus and nitrogen composition of unicellular plants. J. Cellular and Comp. Physiol. 5:55-74.

36. Ketchum, B. H. and A. C. Redfield. 1949. Some physical and chemical characteristics of algae growth in mass culture. J. Cellular and Comp. Physiol. 33:281-299.

37. Kok, B. 1953. Experiments on photosynthesis by Chlorella in flashing light. In Algal Culture from Laboratory to Pilot Plant. J. S. Burlew (ed.). Publ. 600, Carnegie Inst. of Wash. pp. 63-75.

38. Leveille, G. A., H. E. Sauberlich and J. W. Shockley. 1962. Protein value and the amino acid deficiencies of various algae for growth of rats and chicks. Jour. of Nutrition, 76:423-428.

39. McKee, J. E. and H. W. Wolf. 1963. Water Quality Criteria. Publication 3A, the Resources Agency of Calif., State Water Quality Control Board.

40. Milner, H. W. 1948. The fatty acids of Chlorella. J. of Biol. Chem. 176:813-817.

41. Monod, J. 1949. The growth of bacteria culture. Ann. Rev. Microbiol. 3:371.

42. North American Aviation, Inc. 1967. A study of the use of biomass systems in water renovation. Final Report.

43. Osterlind, S. 1950. Inorganic carbon sources of green algae. II - Carbonic anhydrase in Scenedesmus quadricauda and Chlorella pyrenoidosa. Physiologic Plantarum. 3:430-434.

44. Oswald, W. J. 1963. Light conversion efficiency of algae grown in sewage. Trans. Amer. Soc. Civil Engrs. Vol. 128 part III: 47-83.

45. Oswald, W. J., D. G. Crosby and C. G. Golueke. 1964. Removal of pesticides and algal growth potential from San Joaquin Valley drainage water (A feasibility study). Unpublished report submitted to the Calif. Dept. of Water Resources.

46. Oswald, W. J. and C. G. Golueke. 1960. Biological transformation of solar energy in Advances in Applied Microbiology. Academic Press Inc., New York. Vol. II: 223-261.

47. Oswald, W. J. and C. G. Golueke. 1968. Harvesting and processing of waste-grown microalgae. In Algae, Man and the Environment. D. F. Jackson (ed.). Proceedings of a Symposim held June 18-30 1967. pp. 371-389.

48. Oswald, W. J. and H. B. Gotaas. 1955. Photosynthesis in sewage treatment. Proceedings - Separate No. 636. Amer. Soc. Civil Engrs.

49. Retovsky in Malek, I. and Z.Fencl. 1966. Theoretical and Methodological Basis of Continuous Culture of Microorganisms. Academic Press. New York.

50. Sawyer, C. N. 1952. Some new aspects of phosphates in relation to lake fertilization. Sewage and Industrial Wastes. Vol. 24, No. 6:768.

51. Schuler, J. F., V. M. Diller, and H. J. Kerslen. 1953. Preferential assimilation of ammonium ion by Chlorella vulgaris. Plant Physiol., 28:299-303.

52. Shelef, G., W. J. Oswald, and C. G. Golueke. Light intensity and nitrogen concentration as growth limiting factors. U. of Calif. Sanit. Engr. Res. Lab. Report No. 68-4.

53. Smith, R. 1967. A compilation of cost information for conventional and advanced wastewater treatment plants and processes, USDI, FWPCA. Cincinnati, Ohio.

54. Spoehr, H. A. and H. W. Milner. 1949. The chemical composition of Chlorella; Effect of environmental conditions. Plant Physiol., 24:120-149.

55. Stumm, W. and J. J. Morgan. 1962. Chemical aspects of coagulation. J. Amer. Water Work Assoc., 54:971.

56. Tenney, M. W., W. F. Echelberger, Jr., R. G. Schuessler, and J. L. Paroni. 1969. Algal flocculation with synthetic organic polyelectrolytes. J. of Applied Microbiol. Vol. 18, No. 6:965-971.

57. Williford, J. W. and D. R. Cardon. 1970. Potential changes in the nitrogen concentration of drainage water during transport. Report No. 4. Agricultural Wastewater Study Group.

58. Witt, V. M. and J. A. Borchardt. 1960. The removal of nitrogen and phosphorus from sewage effluents through the use of algal culture (Scenedesmus and Chlorella in mixed or unialgal cultures). J. Biochem. Microbiol. Technol. Engr. 2:187-203.

59. Zabat, M., W. Oswald, C. Golueke, and H. Gee. 1970. Kinetics of algal systems in waste treatment, phosphorus as a growth limiting factor. SERL Publication, University of California, Berkeley.

PUBLICATIONS

SAN JOAQUIN PROJECT, FIREBAUGH, CALIFORNIA

1968

"Is Treatment of Agricultural Waste Water Possible?"
Louis A. Beck and Percy P. St. Amant, Jr. Presented at Fourth International Water Quality Symposium, San Francisco, California, August 14, 1968; published in the proceedings of the meeting.

1969

"Biological Denitrification of Wastewaters by Addition of Organic Materials"
Perry L. McCarty, Louis A. Beck, and Percy P. St. Amant, Jr. Presented at the 24th Annual Purdue Industrial Waste Conference, Purdue University, Lafayette, Indiana. May 6, 1969.

"Comparison of Nitrate Removal Methods"
Louis A. Beck, Percy P. St. Amant, Jr., and Thomas A. Tamblyn. Presented at Water Pollution Control Federation Meeting, Dallas, Texas. October 9, 1969.

"Effect of Surface/Volume Relationship, CO_2 Addition, Aeration, and Mixing on Nitrate Utilization by Scenedesmus Cultures in Subsurface Agricultural Waste Waters"
Randall L. Brown and James F. Arthur. Proceedings of the Eutrophication-Biostimulation Assessment Workshop, Berkeley, California. June 19-21, 1969.

"Nitrate Removal Studies at the Interagency Agricultural Waste Water Treatment Center, Firebaugh, California"
Percy P. St. Amant, Jr., and Louis A. Beck. Presented at 1969 Conference, California Water Pollution Control Association, Anaheim, California, and published in the proceedings of the meeting. May 9, 1969.

"Research on Methods of Removing Excess Plant Nutrients from Water"
Percy P. St. Amant, Jr., and Louis A. Beck. Presented at 158th National Meeting and Chemical Exposition, American Chemical Society, New York, New York. September 8,.1969.

"The Anaerobic Filter for the Denitrification of Agricultural Subsurface Drainage"
T. A. Tamblyn and B. R. Sword. Presented at the 24th Purdue Industrial Waste Conference, Lafayette, Indiana. May 5-8, 1969.

SAN JOAQUIN PROJECT, FIREBAUGH, CALIFORNIA

1969

"Nutrients in Agricultural Tile Drainage"
W. H. Pierce, L. A. Beck and L. R. Glandon. Presented at the 1969 Winter Meeting of the American Society of Agricultural Engineers, Chicago, Illinois. December 9-12, 1969.

"Treatment of High Nitrate Waters"
Percy P. St. Amant, Jr., and Perry L. McCarty. Presented at Annual Conference, American Water Works Association, San Diego, California. May 21, 1969. American Water Works Association Journal. Vol. 61. No. 12. December 1969. pp. 659-662.

The following papers were presented at the National Fall Meeting of the American Geophysical Union, Hydrology Section, San Francisco, California. December 15-18, 1969. They are published in Collected Papers Regarding Nitrates in Agricultural Waste Water. USDI, FWQA, #13030 ELY December 1969.

"The Effects of Nitrogen Removal on the Algal Growth Potential of San Joaquin Valley Agricultural Tile Drainage Effluents"
Randall L. Brown, Richard C. Bain, Jr. and Milton G. Tunzi.

"Harvesting of Algae Grown in Agricultural Wastewaters"
Bruce A. Butterfield and James R. Jones.

"Monitoring Nutrients and Pesticides in Subsurface Agricultural Drainage"
Lawrence R. Glandon, Jr., and Louis A. Beck.

"Combined Nutrient Removal and Transport System for Tile Drainage from the San Joaquin Valley"
Joel C. Goldman, James F. Arthur, William J. Oswald, and Louis A. Beck.

"Desalination of Irrigation Return Waters"
Bryan R. Sword.

"Bacterial Denitrification of Agricultural Tile Drainage"
Thomas A. Tamblyn, Perry L. McCarty and Percy P. St. Amant.

"Algal Nutrient Responses in Agricultural Wastewater"
James F. Arthur, Randall L. Brown, Bruce A. Butterfield, Joel C. Goldman.

1 Accession Number	2 Subject Field & Group	SELECTED WATER RESOURCES ABSTRACTS INPUT TRANSACTION FORM
	05 D	

5 Organization

Department of Water Resources
San Joaquin District
Fresno, California

6 Title

REMOVAL OF NITRATE BY AN ALGAL SYSTEM

10 Author(s)

Brown, Randall L.

16 Project Designation

13030 ELY

21 Note

Available from Department of Water Resources
Post Office Box 2385
Fresno, California 93723

22 Citation

Agricultural Wastewater Studies
Report No. 13030 ELY 4/71 - 7
Pages 132, Figures 58, Tables 27, References 59

23 Descriptors (Starred First)

*Agricultural Wastes, *Water Pollution Control, Biological Treatment, Nitrates, Treatment Facilities

25 Identifiers (Starred First)

*Algae Stripping, Scenedesmus, Algal Growth and Harvesting

27 Abstract

An algal system consisting of algae growth, harvesting and disposal was evaluated as a possible means of removing nitrate-nitrogen from subsurface agricultural drainage in the San Joaquin Valley of California. The study of this assimilatory nitrogen removal process was initiated to determine optimum conditions for growth of the algal biomass, seasonal variations in assimilation rates, and methods of harvesting and disposal of the algal product. A secondary objective of the study was to obtain preliminary cost estimates and process design.

The growth studies showed that about 75 to 90 percent of the 20 mg/l influent nitrogen was assimilated by shallow (12-inch culture depth) algal cultures receiving 2 to 3 mg/l additional iron and phosphorus and a mixture of 5 percent CO_2. Theoretical hydraulic detention times required for these assimilation rates varied from 5 to 16 days, depending on the time of the year. The total nitrogen removal by the algal system, assuming 95 percent removal of the algal cells, ranged from 70 to 85 percent of the influent nitrogen.

The most economical and effective algal harvesting system tested was flocculation and sedimentation followed by filtration of the sediment. The algal cake from the vacuum filter, containing about 20 percent solids, was then air- or flash-dried to about 90 percent solids. The market value for this product as a protein supplement was estimated to be about $80 to $100 per ton.

Abstractor Brown	Institution Department of Water Resources

WR:102 (REV JULY 1969)
WRSIC

SEND TO: WATER RESOURCES SCIENTIFIC INFORMATION CENTER
U S DEPARTMENT OF THE INTERIOR
WASHINGTON, D. C 20240

* GPO: 1969—359-339

REMOVAL OF NITRATE BY AN ALGAL SYSTEM